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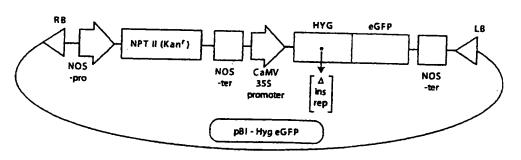
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(54) Title: TARGETED CHROMOSOMAL GENOMIC ALTERATIONS IN PLANTS USING MODIFIED SINGLE STRANDED OLIGONUCLEOTIDES



(57) Abstract: Presented are methods and compositions for targeted chromosomal genomic alterations with modified single-stranded oligonucleotides. The oligonucleotides of the invention have modified nuclease-resisant termini comprising LNA, phosphorothioate linkages or 2'-O-Me base analogues or combinations of such modifications.



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## TARGETED CHROMOSOMAL GENOMIC ALTERATIONS IN PLANTS USING MODIFIED SINGLE STRANDED OLIGONUCLEOTIDES

#### Field Of The Invention

The technical field of the invention is oligonucleotide-directed repair or alteration of plant genetic information using novel chemically modified oligonucleotides.

#### **Background Of The Invention**

A number of methods have been developed specifically to alter the genomic information of plants. These methods generally include the use of vectors such as, for example, T-DNA, carrying nucleic acid sequences encoding partial or complete portions of a particular protein which is expressed in a cell or tissue to effect the alteration. The expression of the particular protein then results in the desired phenotype. See, for example, United States Patent 4,459,355 which describes a method for transforming plants with a DNA vector and United States Patent 5,188,642 which describes cloning or expression vectors containing a transgenic DNA sequence which when expressed in plants confers resistance to the herbicide glyphosate. The use of such transgene-containing vectors adds one or more exogenous copies of a gene in a usually random fashion at one or more integration sites of the plant's genome at some variable frequency. The introduced gene may be foreign or may be derived from the host plant. Any gene which was originally present in the genome, which may be, for example, a normal allelic variant, mutated, defective, and/or functional copy of the introduced gene, is retained in the genome of the host plant.

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These methods of gene alteration are problematic in that complications which can compromise the vigor, productivity, yield, etc. of the plant may result. One such problem is that insertion of exogenous nucleic acid at random location(s) in the genome can have deleterious effects. The random nature of this insertion and/or the use of exogenous promoters can also cause the timing, location or strength of expression of the introduced transgene to be inappropriate or unpredictable. Another problem with such systems includes the addition of unnecessary and unwanted genetic material to the genome of the recipient, including, for example, T-DNA ends or other vector remnants, exogenous control sequences required to allow production of the transgene protein, which control sequences may be

exogenous or native to the host plant and/or the transgene, and reporter genes or resistance markers. Such remnants and added sequences may have presently unrecognized consequences, for example, involving genetic rearrangements of the recipient genomes. In addition, concerns have been raised with consumption, especially by humans, of plants containing such exogenous genetic material.

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In more recent work, the domain for altering a genome is linked or tethered to the triplex forming domain of the bi-functional oligonucleotide, adding an additional linking or tethering functional domain to the oligonucleotide. See, e.g., Culver et al., <a href="Nature Biotechnology">Nature Biotechnology</a> 17: 989-93 (1999). Such chimeric or triplex forming molecules have distinct structural requirements for each of the different domains of the complete poly- or oligo-nucleotide in order to effect the desired genomic alteration in either episomal or chromosomal targets.

More recently, simpler systems involving poly- or oligo- nucleotides have been described for use in the alteration of genomic DNA. These chimeric RNA-DNA oligonucleotides, requiring contiguous RNA and DNA bases in a double-stranded molecule folded by complementarity into a double hairpin conformation, have been shown to effect single basepair or frameshift alterations, for example, for mutation or repair of plant, animal or fungal genomes. See, for example, WO 99/07865 and U.S. Patent 5,565,350. In the chimeric RNA-DNA oligonucleotide, an uninterrupted stretch of DNA bases within the molecule is required for sequence alteration of the targeted genome while the obligate RNA residues are involved in complex stability. Due to the length, backbone composition, and structural configuration of these chimeric RNA-DNA molecules, they are expensive to synthesize and difficult to purify. Moreover, if the RNA-containing strand of the chimeric RNA-DNA oligonucleotide is designed so as to direct gene alteration, a series of mutagenic reactions resulting in nonspecific base alteration can result. Such a result reduces the utility of such a molecule in methods designed for targeted gene alteration.

Alternatively, other oligo- or poly- nucleotides have been used which require a triplex forming, usually polypurine or polypyrimidine, structural domain which binds to a DNA helical duplex through Hoogsteen interactions between the major groove of the DNA duplex and the oligonucleotide. Such oligonucleotides may have an additional DNA reactive moiety, such as psoralen, covalently linked to the oligonucleotide. These reactive moieties function as effective intercalation agents, stabilize the formation of a triplex and can be mutagenic. Such agents may be required in order to stabilize the triplex forming domain of the oligonucleotide with the DNA double helix if the Hoogsteen interactions from the oligonucleotide/target base composition are insufficient. See, e.g., U.S. Patent 5,422,251. The utility of these oligonucleotides for directing targeted gene alteration is compromised by a high frequency of nonspecific base changes.

Other genes, e.g. CFTR, have been targeted by homologous recombination using duplex fragments having several hundred basepairs. See, e.g., Kunzelmann et al., <u>Gene Ther.</u> 3:859-867 (1996). Similar efforts to target genes by homologous recombination in plants using large fragments of DNA had some success. See Kempin et al., <u>Nature</u> 389:802-803 (1997). However, the efficiency and reproducibility of the published homologous recombination approach in plants has severely limited the widespread use of this method.

Earlier experiments to mutagenize an antibiotic resistance indicator gene by homologous recombination used an unmodified DNA oligonucleotide rather than larger fragments of DNA, wherein the oligonucleotide had no functional domains other than a region of complementary sequence to the target. See Campbell et al., New Biologist 1: 223-227 (1989). These experiments required large concentrations of the oligonucleotide, exhibited a very low frequency of episomal modification of a targeted exogenous plasmid gene not normally found in the cell and have not been reproduced. However, as shown in examples herein, we have observed that an unmodified DNA oligonucleotide can convert a base at low frequency which is detectable using the assay systems described herein.

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Oligonucleotides designed for use in the targeted alteration of genetic information are significantly different from oligonucleotides designed for antisense approaches. For example, antisense oligonucleotides are perfectly complementary to and bind an mRNA strand in order to modify expression of a targeted mRNA and are used at high concentration. As a consequence, they are unable to produce a gene conversion event by either mutagenesis or repair of a defect in the chromosomal DNA of a host genome. Furthermore, the backbone chemical composition used in most oligonucleotides designed for use in antisense approaches renders them inactive as substrates for homologous pairing or mismatch repair enzymes and the high concentrations of oligonucleotide required for antisense applications can be toxic with some types of nucleotide modifications. In addition, antisense oligonucleotides must be complementary to the mRNA and therefore, may not be complementary to the other DNA strand or to genomic sequences that span the junction between intron sequence and exon sequence.

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Artificial chromosomes can be useful for the screening purposes identified herein. These molecules are man-made linear or circular DNA molecules constructed from essential cis-acting DNA sequence elements that are responsible for the proper replication and partitioning of natural chromosomes (Murray et al., 1983). The essential elements are: (1) Autonomous Replication Sequences (ARS), (2) Centromeres, and (3) Telomeres.

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Yeast artificial chromosomes (YACs) allow large segments of genomic DNA to be cloned and modified (Burke et al., Science 236:806; Peterson et al., Trends Genet. 13:61 (1997); Choi, et al., Nat.

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Genet., 4:117-223 (1993), Davies, et al., Biotechnology 11:911-914 (1993), Matsuura, et al., Hum. Mol. Genet., 5:451-459 (1996), Peterson et al., Proc. Natl. Acad. Sci., 93:6605-6609 (1996); and Schedl, et al., Cell, 86:71-82 (1996)). Other vectors also have been developed for the cloning of large segments of genomic DNA, including cosmids, and bacteriophage P1 (Stemberg et al., Proc. Natl. Acad. Sci. U.S.A., 87:103-107 (1990)). YACs have certain advantages over these alternative large capacity cloning vectors (Burke et al., Science, 236:806-812 (1987)). The maximum insert size is 35-30 kb for cosmids, and 100 kb for bacteriophage P1, both of which are much smaller than the maximal insert size for a YAC.

An alternative to YACs are cloning systems based on the *E. coli* fertility factor that have been developed to construct large genomic DNA insert libraries. They are bacterial artificial chromosomes (BACs) and P-1 derived artificial chromosomes (PACs) (Mejia et al., Genome Res. 7:179-186 (1997); Shizuya et al., Proc. Natl. Acad. Sci. 89:8794-8797 (1992); loannou et al., Nat. Genet., 6:84-89 (1994); Hosoda et al., Nucleic Acids Res. 18:3863 (1990)). BACs are based on the *E. coli* fertility plasmid (F factor); and PACs are based on the bacteriophage P1. These vectors propagate at a very low copy number (1-2 per cell) enabling genomic inserts up to 300 kb in size to be stably maintained in recombination deficient hosts. The PACs and BACs are circular DNA molecules that are readily isolated from the host genomic background by classical alkaline lysis (Birnboim et al., Nucleic Acids Res. 7:1513-1523 (1979)). In addition, BACs have been developed for transformation of plants with high-molecular weight DNA using the T-DNA system (Hamilton, Gene 24:107-116 (1997); Frary & Hamilton, Transgenic Res. 10: 121-132 (2001)).

A need exists for simple, inexpensive oligonucleotides capable of producing targeted alteration of genetic material such as those described herein as well as methods to identify optimal oligonucleotides that accurately and efficiently alter target DNA.

#### **Summary Of The Invention**

Novel, modified single-stranded nucleic acid molecules that direct gene alteration in plants are identified and the efficiency of alteration is analyzed both *in vitro* using a cell-free extract assay and *in vivo* using a yeast system and a plant system. The alteration in an oligonucleotide of the invention may comprise an insertion, deletion, substitution, as well as any combination of these. Site specific alteration of DNA is not only useful for studying function of proteins *in vivo*, but it is also useful for creating plants with desired phenotypes, including, for example, environmental stress tolerance, improved nutritional value, herbicide resistance, disease resistance, modified oil production, modified starch production, and altered floral morphology including selective sterility. As described herein,

oligonucleotides of the invention target directed specific gene alterations in genomic double-stranded DNA in cells. The target genomic DNA can be nuclear chromosomal DNA as well as plastid or mitochondrial chromosomal DNA. The target DNA can also be a transgene present in the plant cell, including; for example, a previously introduced T-DNA. For screening purposes, the target plant DNA can also be extrachromosomal DNA present in plant or non-plant cells in various forms including, e.g., mammalian artificial chromosomes (MACs), PACs from P-1 vectors, yeast artificial chromosomes (YACs), bacterial artificial chromosomes (BACs), plant artificial chromosomes (PLACs), as well as episomal DNA, including episomal DNA from an exogenous source such as a plasmid or recombinant vector. Many of these artificial chromosome constructs containing plant DNA can be obtained from a variety of sources, including, e.g., the Arabidopsis Biological Resource Center (ABRC) at the Ohio State University, and the Rice Genome Research Program at the MAFF DNA bank in Ibaraki, Japan. The target DNA may be transcriptionally silent or active. In a preferred embodiment, the target DNA to be altered is the non-transcribed strand of a genomic DNA duplex. In a more preferred embodiment, the target DNA to be altered is the non-transcribed strand of a transcribed gene of a genomic DNA duplex.

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The low efficiency of targeted gene alteration obtained using unmodified DNA oligonucleotides is believed to be largely the result of degradation by nucleases present in the reaction mixture or the target cell. Although different modifications are known to have different effects on the nuclease resistance of oligonucleotides or stability of duplexes formed by such oligonucleotides (see, e.g., Koshkin et al., J. Am. Chem. Soc., 120:13252-3), we have found that it is not possible to predict which of any particular known modification would be most useful for any given alteration event, including for the construction of gene alteration oligonucleotides, because of the interaction of different as yet unidentified proteins during the gene alteration event. Herein, a variety of nucleic acid analogs have been developed that increase the nuclease resistance of oligonucleotides that contain them, including, e.g., nucleotides containing phosphorothioate linkages or 2'-O-methyl analogs. We recently discovered that single-stranded DNA oligonucleotides modified to contain 2'-O-methyl RNA nucleotides or phosphorothioate linkages can enable specific alteration of genetic information at a higher level than either unmodified single-stranded DNA or a chimeric RNA/DNA molecule. See, for example, copending applications United States application no. 60/208,538, United States application no. 60/244,989, United States application no. 09/818,875, international application no. PCT/US01/09761 and Gamper et al., Nucleic Acids Research 28: 4332-4339 (2000), the disclosures of which are incorporated herein in their entirety by reference. We also found that additional nucleic acid analogs which increase the nuclease resistance of oligonucleotides that contain them, including, e.g., "locked nucleic acids" or "LNAs", xylo-

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LNAs and L-ribo-LNAs; see, for example, Wengel & Nielsen, WO 99/14226; Wengel, WO 00/56748; Wengel, WO 00/66604; and Jakobsen & Koshkin, WO 01/25478 also allow specific targeted alteration of genetic information.

The assay allows for determining the optimum length of the oligonucleotide, optimum sequence of the oligonucleotide, optimum position of the mismatched base or bases, optimum chemical modification or modifications, optimum strand targeted for identifying and selecting the most efficient oligonucleotide for a particular gene alteration event by comparing to a control oligonucleotide. Control oligonucleotides may include a chimeric RNA-DNA double hairpin oligonucleotide directing the same gene alteration event, an oligonucleotide that matches its target completely, an oligonucleotide in which all linkages are phosphorothiolated, an oligonucleotide fully substituted with 2'-O-methyl analogs or an RNA oligonucleotide. Such control oligonucleotides either fail to direct a targeted alteration or do so at a lower efficiency as compared to the oligonucleotides of the invention. The assay further allows for determining the optimum position of a gene alteration event within an oligonucleotide, optimum concentration of the selected oligonucleotide for maximum alteration efficiency by systematically testing a range of concentrations, as well as optimization of either the source of cell extract by testing different plants or strains, or testing cells derived from different plants or strains, or plant cell lines. Using a series of single-stranded oligonucleotides, comprising all RNA or DNA residues and various mixtures of the two. several new structures are identified as viable molecules in nucleotide conversion to direct or repair a genomic mutagenic event. When extracts from mammalian, plant and fungal cells are used and are analyzed using a genetic readout assay in bacteria, single-stranded oligonucleotides having one of several modifications are found to be more active than a control RNA-DNA double hairpin chimera structure when evaluated using an in vitro gene repair assay. Similar results are also observed in vivo using yeast, mammalian and plant cells. Molecules containing various lengths of modified bases were found to possess greater activity than unmodified single-stranded DNA molecules.

#### **Detailed Description Of The Invention**

The present invention provides oligonucleotides having chemically modified, nuclease resistant residues, preferably at or near the termini of the oligonucleotides, and methods for their identification and use in targeted alteration of plant genetic material, including gene mutation, targeted gene repair and gene knockout. The oligonucleotides are preferably used for mismatch repair or alteration by changing at least one nucleic acid base, or for frameshift repair or alteration by addition or deletion of at least one nucleic acid base. The oligonucleotides of the invention direct any such alteration,

including gene correction, gene repair or gene mutation and can be used, for example, to introduce a polymorphism or haplotype or to eliminate ("knockout") a particular protein activity. For example, gene alterations that knockout a particular protein activity can be obtained using oligonucleotides designed to convert a codon in the coding region of the protein to a stop codon, thus prematurely terminating translation of the protein. Oligonucleotides that introduce stop codons in the open-reading-frame of the protein are one embodiment of the invention. Generally, oligonucleotides that introduce stop codons early in the open-reading-frame of the protein are preferred. If the open-reading-frame contains more than one methionine, oligonucleotides that introduce stop codons after the second methionine are preferred. Additionally, if the gene exhibits alternative splice sites, oligonucleotides that introduce stop codons in exons after the alternative splice site are preferred. The following table provides examples of codons that can be converted to stop codons by altering a single oligonucleotide. A skilled artisan could readily identify other codons that can be converted to stop codons by altering one, two or three of the base pairs in a given codon. Similarly, a skilled artisan could readily identify codons that can be converted to stop codons by a frameshift mutations that inserts or deletes one or two base pairs in the open-reading-frame. It is also understood that more than one stop codon can be generated in a single open-reading-frame and that these stop codons can be adjacent in the sequence or separated by intervening codons. Where more than one stop codon is introduced into a single open-reading-frame, such alterations can be generated by a single or multiple oligonucleotides and can be generated simultaneously or by sequential mutagenesis of the target nucleic acid.

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Original codons*	Corresponding stop codon
GGA (glycine), AGA (arginine), CGA (arginine), TTA (leucine),	TGA
TCA (serine), TGT (cysteine), TGG (tryptophan), TGC (cysteine)	
AAG (lysine), GAG (glutamate), CAG (glutamine), TTG (leucine),	TAG
TCG (serine), TGG (tryptophan), TAT (cysteine), TAC (tyrosine)	
AAA (lysine), GAA (glutamate), CAA (glutamine), TTA (leucine),	TAA
TCA (serine), TAT (cysteine), TAC (tyrosine)	

<sup>\*</sup>The amino acid encoded by the original codon is shown in parentheses and the base targeted for alteration to convert the codon to the corresponding stop codon is underlined and in bold

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The oligonucleotides of the invention are designed as substrates for homologous pairing and repair enzymes and as such have a unique backbone composition that differs from chimeric RNA-DNA double hairpin oligonucleotides, antisense oligonucleotides, and/or other poly- or oligo-nucleotides used for altering genomic DNA, such as triplex forming oligonucleotides. The single-stranded oligonucleotides described herein are inexpensive to synthesize and easy to purify. In side-by-side comparisons, an optimized single-stranded oligonucleotide comprising modified residues as described herein is significantly more efficient than a chimeric RNA-DNA double hairpin oligonucleotide in directing a base substitution or frameshift mutation in a cell-free extract assay.

We have discovered that single-stranded oligonucleotides having a DNA domain surrounding the targeted base, with the domain preferably central to the poly- or oligo-nucleotide, and having at least one modified end, preferably at the 3' terminal region, are able to alter a target genetic sequence and with an efficiency that is higher than chimeric RNA-DNA double hairpin oligonucleotides disclosed in US Patent 5,565,350. Preferred oligonucleotides of the invention have at least two modified bases on at least one of the termini, preferably the 3' terminus of the oligonucleotide. Oligonucleotides of the invention can efficiently be used to introduce targeted alterations in a genetic sequence of DNA in the presence of human, animal, plant, fungal (including yeast) proteins and in cells of different types including, for example, plant cells, fungal cells including S. cerevisiae, Ustillago maydis, Candida albicans, and mammalian cells. Particularly preferred are cells and cell extracts derived from plants including, for example, experimental model plants such as Chlamydomonas reinhardtii, Physcomitrella patens, and Arabidopsis thaliana in addition to crop plants such as cauliflower (Brassica oleracea), artichoke (Cynara scolymus), fruits such as apples (Malus, e.g. domesticus), mangoes (Mangifera, e.g. indica), banana (Musa, e.g. acuminata), berries (such as currant, Ribes, e.g. rubrum), kiwifruit (Actinidia, e.g. chinensis), grapes (Vitis, e.g. vinifera), bell peppers (Capsicum, e.g. annuum), cherries (such as the sweet cherry, Prunus, e.g. avium), cucumber (Cucumis, e.g. sativus), melons (Cucumis, e.g. melo), nuts (such as walnut, Juglans, e.g. regia; peanut, Arachis hypogeae), orange (Citrus, e.g. maxima), peach (Prunus, e.g. persica), pear (Pyra, e.g. communis), plum (Prunus, e.g. domestica), strawberry (Fragaria, e.g. moschata or vesca), tomato (Lycopersicon, e.g. esculentum); leaves and forage, such as alfalfa (Medicago, e.g. sativa or truncatula), cabbage (e.g. Brassica oleracea), endive (Cichoreum, e.g. endivia), leek (Allium, e.g. porrum), lettuce (Lactuca, e.g. sativa), spinach (Spinacia, e.g. oleraceae), tobacco (Nicotiana, e.g. tabacum); roots, such as arrowroot (Maranta, e.g. arundinacea), beet (Beta, e.g. vulgaris), carrot (Daucus, e.g. carota), cassava (Manihot, e.g. esculenta), turnip (Brassica, e.g. rapa), radish (Raphanus, e.g. sativus), yam (Dioscorea, e.g. esculenta), sweet potato (Ipomoea batatas); seeds, including oilseeds,

such as beans (Phaseolus, e.g. vulgaris), pea (Pisum, e.g. sativum), soybean (Glycine, e.g. max), cowpea (Vigna unguiculata), mothbean (Vigna aconitifolia), wheat (Triticum, e.g. aestivum), sorghum (Sorghum e.g. bicolor), barley (Hordeum, e.g. vulgare), com (Zea, e.g. mays), rice (Oryza, e.g. sativa), rapeseed (Brassica napus), millet (Panicum sp.), sunflower (Helianthus annuus), oats (Avena sativa), chickpea (Cicer, e.g. arietinum); tubers, such as kohlrabi (Brassica, e.g. oleraceae), potato (Solanum, e.g. tuberosum) and the like; fiber and wood plants, such as flax (Linum e.g. usitatissimum), cotton (Gossypium e.g. hirsutum), pine (Pinus sp.), oak (Quercus sp.), eucalyptus (Eucalyptus sp.), and the like and ornamental plants such as turfgrass (Lolium, e.g. rigidum), petunia (Petunia, e.g. x hybrida), hyacinth (Hyacinthus orientalis), carnation (Dianthus e.g. caryophyllus), delphinium (Delphinium, e.g. ajacis), Job's tears (Coix lacryma-jobi), snapdragon (Antirrhinum majus), poppy (Papaver, e.g. nudicaule), lilac (Syringa, e.g. vulgaris), hydrangea (Hydrangea e.g. macrophylla), roses (including Gallicas, Albas, Damasks, Damask Perpetuals, Centifolias, Chinas, Teas and Hybrid Teas) and ornamental goldenrods (e.g. Solidago spp.). Such plant cells can then be used to regenerate whole plants according to methods described herein or any method known in the art. The DNA domain of the oligonucleotides is preferably fully complementary to one strand of the gene target, except for the mismatch base or bases responsible for the gene alteration event(s). On either side of the preferably central DNA domain, the contiguous bases may be either RNA bases or, preferably, are primarily DNA bases. The central DNA domain is generally at least 8 nucleotides in length. The base(s) targeted for alteration in the most preferred embodiments are at least about 8, 9 or 10 bases from one end of the oligonucleotide.

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According to certain embodiments, one or both of the termini of the oligonucleotides of the present invention comprise phosphorothioate modifications, LNA backbone (including LNA derivatives and analogs) modifications, or 2'-O-methyl base analogs, or any combination of these modifications. Oligonucleotides comprising 2'-O-methyl or LNA analogs are a mixed DNA/RNA polymer. The oligonucleotides of the invention are, however, single-stranded and are not designed to form a stable internal duplex structure within the oligonucleotide. The efficiency of gene alteration is surprisingly increased with oligonucleotides having internal complementary sequence comprising phosphorothioate modified bases as compared to 2'-O-methyl modifications. This result indicates that specific chemical interactions are involved between the converting oligonucleotide and the proteins involved in the conversion. The effect of other such chemical interactions to produce nuclease resistant termini using modifications other than LNA (including LNA derivatives or analogs), phosphorothioate linkages, or 2'-O-methyl analog incorporation into an oligonucleotide can not yet be predicted because the proteins

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involved in the alteration process and their particular chemical interaction with the oligonucleotide substituents are not yet known and cannot be predicted.

In the examples, oligonucleotides of defined sequence are provided for alteration of genes in particular plants. Provided the teachings of the instant application, one of skill in the art could readily design oligonucleotides to introduce analogous alterations in homologous genes from any plant." Furthermore, in the tables of these examples, the oligonucleotides of the invention are not limited to the particular sequences disclosed. The oligonucleotides of the invention include extensions of the appropriate sequence of the longer 120 base oligonucleotides which can be added base by base to the smallest disclosed oligonucleotides of 17 bases. Thus the oligonucleotides of the invention include for each correcting change, oligonucleotides of length 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 32, 33, 34, 35, 36, 37, 38, 39, 40, 41, 42, 43, 44, 45, 46, 47, 48, 49, 50, 51, 52, 53, 54, 55, 56, 57, 58, 59, 60, 61, 62, 63, 64, 65, 66, 67, 68, 69, 70, 71, 72, 73, 74, 75, 76, 77, 78, 79, 80, 81, 82, 83, 84, 85, 86, 87, 88, 89, 90, 91, 92, 93, 94, 95, 96, 97, 98, 99, 100, 101, 102, 103, 104, 105, 106, 107, 108, 109, 110, 111, 112, 113, 114, 115, 116, 117, 118, 119, or 120 with further single-nucleotide additions up to the longest sequence disclosed. In some embodiments, longer nucleic acids of up to 240 bases which comprise the sequences disclosed herein may be used. Moreover, the oligonucleotides of the invention do not require a symmetrical extension on either side of the central DNA domain. Similarly, the oligonucleotides of the invention as disclosed in the various tables for alteration of particular plant genes contain phosphorothioate linkages, 2'-O-methyl analog or LNA (including LNA derivatives and analogs) or any combination of these modifications just as the assay oligonucleotides do.

The present invention, however, is not limited to oligonucleotides that contain any particular nuclease resistant modification. Oligonucleotides of the invention may be altered with any combination of additional LNAs (including LNA derivatives and analogs), phosphorothioate linkages or 2'-O-methyl analogs to maximize conversion efficiency. For oligonucleotides of the invention that are longer than about 17 to about 25 bases in length, internal as well as terminal region segments of the backbone may be altered. Alternatively, simple fold-back structures at each end of a oligonucleotide or appended end groups may be used in addition to a modified backbone for conferring additional nuclease resistance.

The different oligonucleotides of the present invention preferably contain more than one of the aforementioned backbone modifications at each end. In some embodiments, the backbone modifications are adjacent to one another. However, the optimal number and placement of backbone modifications for any individual oligonucleotide will vary with the length of the oligonucleotide and the particular type of backbone modification(s) that are used. If constructs of identical sequence having

phosphorothioate linkages are compared, 2, 3, 4, 5, or 6 phosphorothioate linkages at each end are preferred. If constructs of identical sequence having 2'-O-methyl base analogs are compared, 1, 2, 3 or 4 analogs are preferred. The optimal number and type of backbone modifications for any particular oligonucleotide useful for altering target DNA may be determined empirically by comparing the alteration efficiency of the oligonucleotide comprising any combination of the modifications to a control molecule of comparable sequence using any of the assays described herein. The optimal position(s) for oligonucleotide modifications for a maximally efficient altering oligonucleotide can be determined by testing the various modifications as compared to control molecule of comparable sequence in one of the assays disclosed herein. In such assays, a control molecule includes, e.g., a completely 2'-O-methyl substituted molecule, a completely complementary oligonucleotide, or a chimeric RNA-DNA double hairpin.

Increasing the number of phosphorothioate linkages, LNAs or 2'-O-methyl bases beyond the preferred number generally decreases the gene repair activity of a 25 nucleotide long oligonucleotide. Based on analysis of the concentration of oligonucleotide present in the extract after different time periods of incubation, it is believed that the terminal modifications impart nuclease resistance to the oligonucleotide thereby allowing it to survive within the cellular environment. However, this may not be the only possible mechanism by which such modifications confer greater efficiency of conversion. For example, as disclosed herein, certain modifications to oligonucleotides confer a greater improvement to the efficiency of conversion than other modifications.

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Efficiency of conversion is defined herein as the percentage of recovered substrate molecules that have undergone a conversion event. Depending on the nature of the target genetic material, e.g. the genome of a cell, efficiency could be represented as the proportion of cells or clones containing an extrachromosomal element that exhibit a particular phenotype. Alternatively, representative samples of the target genetic material can be sequenced to determine the percentage that have acquired the desire change. The oligonucleotides of the invention in different embodiments can alter DNA two, three, four, five, six, seven, eight, nine, ten, twelve, fifteen, twenty, thirty, and fifty or more fold more than control oligonucleotides. Such control oligonucleotides are oligonucleotides with fully phosphorothiolated linkages, oligonucleotides that are fully substituted with 2'-O-methyl analogs, a perfectly matched oligonucleotide that is fully complementary to a target sequence or a chimeric DNA-RNA double hairpin oligonucleotide such as disclosed in US Patent 5,565,350.

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In addition, for a given oligonucleotide length, additional modifications interfere with the ability of the oligonucleotide to act in concert with the cellular recombination or repair enzyme machinery

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which is necessary and required to mediate a targeted substitution, addition or deletion event in DNA. For example, fully phosphorothiolated or fully 2-O-methylated molecules are inefficient in targeted gene alteration.

The oligonucleotides of the invention as optimized for the purpose of targeted alteration of genetic material, including gene knockout or repair, are different in structure from antisense oligonucleotides that may possess a similar mixed chemical composition backbone. The oligonucleotides of the invention differ from such antisense oligonucleotides in chemical composition, structure, sequence, and in their ability to alter genomic DNA. Significantly, antisense oligonucleotides fail to direct targeted gene alteration. The oligonucleotides of the invention may target either strand of DNA and can include any component of the genome including, for example, intron and exon sequences. The preferred embodiment of the invention is a modified oligonucleotide that binds to the non-transcribed strand of a genomic DNA duplex. In other words, the preferred oligonucleotides of the invention target the sense strand of the DNA, i.e. the oligonucleotides of the invention are complementary to the non-transcribed strand of the target duplex DNA. The sequence of the non-transcribed strand of a DNA duplex is found in the mRNA produced from that duplex, given that mRNA uses uracil-containing nucleotides in place of thymine-containing nucleotides.

Moreover, the initial observation that single-stranded oligonucleotides comprising these modifications and lacking any particular triplex forming domain have reproducibly enhanced gene afteration activity in a variety of assay systems as compared to a chimeric RNA-DNA double-stranded hairpin control or single-stranded oligonucleotides comprising other backbone modifications was surprising. The single-stranded molecules of the invention totally lack the complementary RNA binding structure that stabilizes a normal chimeric double-stranded hairpin of the type disclosed in U.S. Patent 5,565,350 yet is more effective in producing targeted base conversion as compared to such a chimeric RNA-DNA double-stranded hairpin. In addition, the molecules of the invention lack any particular triplex forming domain involved in Hoogsteen interactions with the DNA double helix and required by other known oligonucleotides in other oligonucleotide-dependant gene conversion systems. Although the lack of these functional domains was expected to decrease the efficiency of an alteration in a sequence, just the opposite occurs: the efficiency of sequence alteration using the modified oligonucleotides of the invention is higher than the efficiency of sequence alteration using a chimeric RNA-DNA hairpin targeting the same sequence alteration. Moreover, the efficiency of sequence alteration or gene conversion directed by an unmodified oligonucleotide is many times lower as compared to a control chimeric RNA-DNA molecule or the modified oligonucleotides of the invention targeting the

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same sequence alteration. Similarly, molecules containing at least 3.2'-O-methyl base analogs are about four to five fold less efficient as compared to an oligonucleotide having the same number of phosphorothioate linkages.

The oligonucleotides of the present invention for alteration of a single base are about 17 to about 121 nucleotides in length, preferably about 17 to about 74 nucleotides in length. Most preferably, however, the oligonucleotides of the present invention are at least about 25 bases in length, unless there are self-dimerization structures within the oligonucleotide. If the oligonucleotide has such an unfavorable structure, lengths longer than 35 bases are preferred. Oligonucleotides with modified ends both shorter and longer than certain of the exemplified, modified oligonucleotides herein function as gene repair or gene knockout agents and are within the scope of the present invention.

Once an oligomer is chosen, it can be tested for its tendency to self-dimerize, since self-dimerization may result in reduced efficiency of alteration of genetic information. Checking for self-dimerization tendency can be accomplished manually or, preferably, using a software program. One such program is Oligo Analyzer 2.0, available through Integrated DNA Technologies (Coralville, IA 52241) (http://www.idtdna.com); this program is available for use on the world wide web at

http://www.idtdna.com/program/oligoanalyzer/

oligoanalyzer.asp.

For each oligonucleotide sequence input into the program, Oligo Analyzer 2.0 reports possible self-dimerized duplex forms, which are usually only partially duplexed, along with the free energy change associated with such self-dimerization. Delta G-values that are negative and large in magnitude, indicating strong self-dimerization potential, are automatically flagged by the software as "bad". Another software program that analyzes oligomers for pair dimer formation is Primer Select from DNASTAR, Inc., 1228 S. Park St., Madison, WI 53715, Phone: (608) 258-7420 (http://www.dnastar.com/products/PrimerSelect.html).

If the sequence is subject to significant self-dimerization, the addition of further sequence flanking the "repair" nucleotide can improve gene correction frequency.

Generally, the oligonucleotides of the present invention are identical in sequence to one strand of the target DNA, which can be either strand of the target DNA, with the exception of one or more targeted bases positioned within the DNA domain of the oligonucleotide, and preferably toward the middle between the modified terminal regions. Preferably, the difference in sequence of the oligonucleotide as compared to the targeted genomic DNA is located at about the middle of the oligonucleotide sequence. In a preferred embodiment, the oligonucleotides of the invention are complementary to the non-transcribed

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strand of a duplex. In other words, the preferred oligonucleotides target the sense strand of the DNA, i.e. the oligonucleotides of the invention are preferably complementary to the strand of the target DNA the sequence of which is found in the mRNA.

The oligonucleotides of the invention can include more than a single base change. In an oligonucleotide that is about a 70-mer, with at least one modified residue incorporated on the ends, as disclosed herein, multiple bases can be simultaneously targeted for change. The target bases may be up to 27 nucleotides apart and may not be changed together in all resultant plasmids in all cases. There is a frequency distribution such that the closer the target bases are to each other in the central DNA domain within the oligonucleotides of the invention, the higher the frequency of change in a given cell. Target bases only two nucleotides apart are changed together in every case that has been analyzed. The farther apart the two target bases are, the less frequent the simultaneous change. Thus, oligonucleotides of the invention may be used to repair or alter multiple bases rather than just one single base. For example, in a 74-mer oligonucleotide having a central base targeted for change, a base change event up to about 27 nucleotides away can also be effected. The positions of the altering bases within the oligonucleotide can be optimized using any one of the assays described herein. Preferably, the altering bases are at least about 8 nucleotides from one end of the oligonucleotide.

The oligonucleotides of the present invention can be introduced into cells by any suitable means. According to certain preferred embodiments, the modified oligonucleotides may be used alone. Suitable means, however, include the use of polycations, cationic lipids, liposomes, polyethylenimine (PEI), electroporation, biolistics, microinjection and other methods known in the art to facilitate cellular uptake. For plant cells, biolistic or particle bombardment methods are typically used. According to certain preferred embodiments of the present invention, isolated plant cells are treated in culture according to the methods of the invention, to mutate or repair a target gene. Alternatively, plant target DNA may be modified *in vitro* or in another cell type, including for example, yeast or bacterial cells and then introduced into a plant cell as, for example, a T-DNA. Plant cells thus modified may be used to regenerate the whole organism as, for example, in a plant having a desired targeted genomic change. In other instances, targeted genomic alteration, including repair or mutagenesis, may take place *in vivo* following direct administration of the modified, single-stranded oligonucleotides of the invention to a subject.

The single-stranded, modified oligonucleotides of the present invention have numerous applications as gene repair, gene modification, or gene knockout agents. Such oligonucleotides may be advantageously used, for example, to introduce or correct multiple point mutations. Each mutation leads to the addition, deletion or substitution of at least one base pair. The methods of the present invention

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offer distinct advantages over other methods of altering the genetic makeup of an organism, in that only the individually targeted bases are altered. No additional foreign DNA sequences are added to the genetic complement of the organism. Such agents may, for example, be used to develop plants with improved traits by rationally changing the sequence of selected genes in isolated cells and using these modified cells to regenerate whole plants having the altered gene. See, e.g., U.S. Patent 6,046,380 and U.S. Patent 5,905,185 incorporated herein by reference. Such plants produced using the compositions of the invention lack additional undesirable selectable markers or other foreign DNA sequences. Targeted base pair substitution or frameshift mutations introduced by an oligonucleotide in the presence of a cell-free extract also provides a way to modify the sequence of extrachromosomal elements, including, for example, plasmids, cosmids and artificial chromosomes. The oligonucleotides of the invention also simplify the production of plants having particular modified or inactivated genes. Altered plant model systems such as those produced using the methods and oligonucleotides of the invention are invaluable in determining the function of a gene and in evaluating drugs. The oligonucleotides and methods of the present invention may also be used to introduce molecular markers, including, for example, SNPs, RFLPs, AFLPs and CAPs.

The purified oligonucleotide compositions may be formulated in accordance with routine procedures depending on the target. For example, purified oligonucleotide can be used directly in a standard reaction mixture to introduce alterations into targeted DNA *in vitro* or where cells are the target as a composition adapted for bathing cells in culture or for microinjection into cells in culture. The purified oligonucleotide compositions may also be provided on coated microbeads for biolistic delivery into plant cells. Where necessary, the composition may also include a solubilizing agent. Generally, the ingredients will be supplied either separately or mixed together in single-use form, for example, as a dry, lyophilized powder or water-free concentrate. In general, dosage required for efficient targeted gene alteration will range from about 0.001 to 50,000 µg/kg target tissue, preferably between 1 to 250 µg/kg, and most preferably at a concentration of between 30 and 60 micromolar.

For cell administration, direct injection into the nucleus, biolistic bombardment, electroporation, liposome transfer and calcium phosphate precipitation may be used. In yeast, lithium acetate or spheroplast transformation may also be used. In a preferred method, the administration is performed with a liposomal transfer compound, e.g., DOTAP (Boehringer-Mannheim) or an equivalent such as lipofectin. The amount of the oligonucleotide used is about 500 nanograms in 3 micrograms of DOTAP per 100,000 cells. For electroporation, between 20 and 2000 nanograms of oligonucleotide per million cells to be electroporated is an appropriate range of dosages which can be increased to improve

efficiency of genetic alteration upon review of the appropriate sequence according to the methods described herein. For biolistic delivery, microbeads are generally coated with resuspended oligonucleotides, which range of oligonucleotide to microbead concentration can be similarly adjusted to improve efficiency as determined using one of the assay methods described herein, starting with about 0.05 to 1 microgram of oligonucleotide to 25 microgram of 1.0 micrometer gold beads or similar microcarrier.

Another aspect of the invention is a kit comprising at least one oligonucleotide of the invention. The kit may comprise an additional reagent or article of manufacture. The additional reagent or article of manufacture may comprise a delivery mechanism, cell extract, a cell, or a plasmid, such as one of those disclosed in the Figures herein, for use in an assay of the invention. Alternatively, the invention includes a kit comprising an isogenic set of cells in which each cell in the kit comprises a different altered amino acid for a target protein encoded by a targeted altered gene within the cell produced according to the methods of the invention.

Figure 1. Flow diagram for the generation of modified single-stranded oligonucleotides.

#### **Brief Description Of The Drawings**

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The upper strands of chimeric oligonucleotides I and II are separated into pathways resulting in the generation of single-stranded oligonucleotides that contain (A) 2'-O-methyl RNA nucleotides or (B) phosphorothioate linkages. Fold changes in repair activity for correction of kans in the HUH7 cell-free extract are presented in parenthesis. HUH7 cells are described in Nakabayashi et al., Cancer Research 42: 3858-3863 (1982). Each single-stranded oligonucleotide is 25 bases in length and contains a G residue mismatched to the complementary sequence of the kans gene. The numbers 3, 6, 8, 10, 12 and 12.5 respectively indicate how many phosphorothioate linkages (S) or 2'-O-methyl RNA nucleotides (R) are at each end of the molecule. Hence oligo 12S/25G contains an all phosphorothicate backbone, displayed as a dotted line. Smooth lines indicate DNA residues, wavy lines indicate 2'-O-methyl RNA residues and the carat indicates the mismatched base site (G). Figure 1(C) provides a schematic plasmid indicating the sequence of the kan chimeric double-stranded hairpin oligonucleotide (left) and the sequence the tet chimeric double-stranded hairpin oligonucleotide used in other experiments. Figure 1(D) provides a flow chart of a kan experiment in which a chimeric double-stranded hairpin oligonucleotide is used.

Figure 2. Genetic readout system for correction of a point mutation in plasmid pKsm4021. A mutant kanamycin gene harbored in plasmid pKm4021 is the target for correction by oligonucleotides.

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The mutant G is converted to a C by the action of the oligo. Corrected plasmids confer resistance to kanamycin in *E.coli* (DH10B) after electroporation leading to the genetic readout and colony counts.

Figure 3: Target plasmid and sequence correction of a frameshift mutation by chimeric and single-stranded oligonucleotides. (A) Plasmid pT<sup>s</sup>Δ208 contains a single base deletion mutation at position 208 rendering it unable to confer tet resistance. The target sequence presented below indicates the insertion of a T directed by the oligonucleotides to re-establish the resistant phenotype. (B) DNA sequence confirming base insertion directed by Tet 3S/25G; the yellow highlight indicates the position of frameshift repair.

Figure 4. DNA sequences of representative kan' colonies. Confirmation of sequence alteration directed by the indicated molecule is presented along with a table outlining codon distribution. Note that 10S/25G and 12S/25G elicit both mixed and unfaithful gene repair. The number of clones sequenced is listed in parentheses next to the designation for the single-stranded oligonucleotide. A plus (+) symbol indicates the codon identified while a figure after the (+) symbol indicates the number of colonies with a particular sequence. TAC/TAG indicates a mixed peak. Representative DNA sequences are presented below the table with yellow highlighting altered residues.

Figure 5. Gene correction in HeLa cells. Representative oligonucleotides of the invention are co-transfected with the pCMVneo(')FIAsH plasmid (shown in Figure 9) into HeLa cells. Ligand is diffused into cells after co-transfection of plasmid and oligonucleotides. Green fluorescence indicates gene correction of the mutation in the antibiotic resistance gene. Correction of the mutation results in the expression of a fusion protein that carries a marker ligand binding site and when the fusion protein binds the ligand, a green fluorescence is emitted. The ligand is produced by Aurora Biosciences and can readily diffuse into cells enabling a measurement of corrected protein function; the protein must bind the ligand directly to induce fluorescence. Hence cells bearing the corrected plasmid gene appear green while "uncorrected" cells remain colorless.

Figure 6. Z-series imaging of corrected cells. Serial cross-sections of the HeLa cell represented in Figure 5 are produced by Zeiss 510 LSM confocal microscope revealing that the fusion protein is contained within the cell.

Figure 7. Hygromycin-eGFP target plasmids. (A) Plasmid pAURHYG(ins)GFP contains a single base insertion mutation between nucleotides 136 and 137, at codon 46, of the Hygromycin B coding sequence (cds) which is transcribed from the constitutive ADH1 promoter. The target sequence presented below indicates the deletion of an A and the substitution of a C for a T directed by the oligonucleotides to re-establish the resistant phenotype. (B) Plasmid pAURHYG(rep)GFP contains a

base substitution mutation introducing a G at nucleotide 137, at codon 46, of the Hygromycin B coding sequence (cds). The target sequence presented below the diagram indicates the amino acid conservative replacement of G with C, restoring gene function.

Figure 8. Oligonucleotides for correction of hygromycin resistance gene. The sequence of the oligonucleotides used in experiments to assay correction of a hygromycin resistance gene are shown. DNA residues are shown in capital letters, RNA residues are shown in lowercase and nucleotides with a phosphorothioate backbone are capitalized and underlined.

Figure 9. pAURNeo(-)FIAsH plasmid. This figure describes the plasmid structure, target sequence, oligonucleotides, and the basis for detection of the gene alteration event by fluorescence.

Figure 10. pYESHyg(x)eGFP plasmid. This plasmid is a construct similar to the pAURHyg(x)eGFP construct shown in Figure 7, except the promoter is the inducible GAL1 promoter. This promoter is inducible with galactose, leaky in the presence of raffinose, and repressed in the presence of dextrose.

Figure 11. pBI-HygeGFP plasmid. This plasmid is a construct based on the plasmids pBI101, pBI 101.2, pBI101.3 or pBI 121 available from Clontech in which HygeGFP replaces the beta-glucuronidase gene of the Clontech plasmids. The different Clontech plasmids vary by a reading frame shift relative to the polylinker, or the presence of the Cauliflower mosaic virus promoter.

The following examples are provided by way of illustration only, and are not intended to limit the scope of the invention disclosed herein.

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# EXAMPLE 1 Assay Method For Base Alteration And Preferred Oligonucleotide Selection

In this example, single-stranded and double-hairpin oligonucleotides with chimeric backbones (see Figure 1 for structures (A and B) and sequences (C and D) of assay oligonucleotides) are used to correct a point mutation in the kanamycin gene of pKsm4021 (Figure 2) or the tetracycline gene of pTsA208 (Figure 3). All kan oligonucleotides share the same 25 base sequence surrounding the target base identified for change, just as all tet oligonucleotides do. The sequence is given in Figures 1C and Figure 1D. Each plasmid contains a functional ampicillin gene. Kanamycin gene function is restored when a G at position 4021 is converted to a C (via a substitution mutation); tetracycline gene function is restored when a deletion at position 208 is replaced by a C (via frameshift mutation). A separate plasmid, pAURNeo(-)FIAsH (Figure 9), bearing the kans gene is used in the cell culture experiments. This plasmid was constructed by inserting a synthetic expression cassette containing a neomycin phosphotransferase

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(kanamycin resistance) gene and an extended reading frame that encodes a receptor for the FIAsH ligand into the pAUR123 shuttle vector (Panvera Corp., Madison, WI). The resulting construct replicates in *S. cerevisiae* at low copy number, confers resistance to aureobasidinA and constitutively expresses either the Neo+/FIAsH fusion product (after alteration) or the truncated Neo-/FIAsH product (before alteration) from the ADH1 promoter. By extending the reading frame of this gene to code for a unique peptide sequence capable of binding a small ligand to form a fluorescent complex, restoration of expression by correction of the stop codon can be detected in real time using confocal microscopy.

Additional constructs can be made to test additional gene alteration events or for specific use in different expression systems. For example, alternative comparable plant plasmids or integration vectors such as, e.g. those based on T-DNA, can be constructed for stable expression in plant cells according to the disclosures herein. Such constructs would use a plant specific promoter such as, e.g., cauliflower mosaic virus 35S promoter, to replace the promoters directing expression of the neo, hyg or aureobasidinA resistance gene disclosed herein, including for example, in Figures 7B, 9 and 10 herein. Moreover, the green fluorescent protein (GFP) sequence used herein may be modified to increase expression in plant cells such as Arabidopsis and the other plants disclosed herein as described in Haseloff et al., Proc. Natl.Acad. Sci. 94(6): 2122-7 (1997), Rouwendal et al. Plant Mol. Biol. 33(6): 989-99 (1997) and Hu et al. FEBS Lett. 369(2-3): 331-4 (1995). Codon usage for optimal expression of GFP in plants results from increasing the frequency of codons with a C or a G in the third position from 32 to about 60%. Specific constructs are disclosed and can be used as follows with such plant specific alterations.

We also construct three mammalian expression vectors, pHyg(rep)eGFP, pHyg(Δ)eGFP, pHyg(ins)eGFP, that contain a substitution mutation at nucleotide 137 of the hygromycin-B coding sequence. (rep) indicates a T137→G replacement, (Δ) represents a deletion of the G137 and (ins) represents an A insertion between nucleotides 136 and 137. All point mutations create a nonsense termination codon at residue 46. We use pHYGeGFP plasmid (Invitrogen, CA) DNA as a template to introduce the mutations into the hygromycin-eGFP fusion gene by a two step site-directed mutagenesis PCR protocol. First, we generate overlapping 5' and a 3' amplicons surrounding the mutation site by PCR for each of the point mutation sites. A 215 bp 5' amplicon for the (rep), (Δ) or (ins) was generated by polymerization from oligonucleotide primer HygEGFPf (5'-AATACGACTCACTATAGG-3') to primer Hygrepr (5'GACCTATCCACGCCCTCC-3'), HygΔr (5'-GACTATCCACGCCCTCC-3'), or Hyginsr (5'-GACATTATCCACGCCCTCC-3'), respectively. We generate a 300bp 3' amplicon for the (rep), (Δ) or (ins) by polymerization from oligonucleotide primers Hygrepf (5'-CTGGGATAGGTCCTGCGG-3'), HygΔf

(5'-CGTGGATAGTCCTGCGG-3'), Hyginsf (5'-CGTGGATAATGTCCTGCGG-3'), respectively to primer HygEGFPr (5'-AAATCACGCCATGTAGTG-3'). We mix 20 ng of each of the resultant 5' and 3' overlapping amplicon mutation sets and use the mixture as a template to amplify a 523 bp fragment of the Hygromycin gene spanning the KpnI and RsrII restriction endonuclease sites. We use the Expand PCR system (Roche) to generate all amplicons with 25 cycles of denaturing at 94°C for 10 seconds, annealing at 55°C for 20 seconds and elongation at 68°C for 1 minute. We digest 10 µg of vector pHYGeGFP and 5 µg of the resulting fragments for each mutation with KpnI and RsrII (NEB) and gel purify the fragment for enzymatic ligation. We ligate each mutated insert into pHYGeGFP vector at 3:1 molar ratio using T4 DNA ligase (Roche). We screen clones by restriction digest, confirm the mutation by Sanger dideoxy chain termination sequencing and purify the plasmid using a Qiagen maxiprep kit.

Oligonucleotide synthesis and cells. Chimeric oligonucleotides and single-stranded oligonucleotides (including those with the indicated modifications) are synthesized using available phosphoramidites on controlled pore glass supports. After deprotection and detachment from the solid support, each oligonucleotide is gel-purified using, for example, procedures such as those described in Gamper et al., Biochem. 39, 5808-5816 (2000) and the concentrations determined spectrophotometrically (33 or 40 µg/ml per A<sub>260</sub> unit of single-stranded or hairpin oligomer). HUH7 cells are grown in DMEM, 10% FBS, 2mM glutamine, 0.5% pen/strep. The *E.coli* strain, DH10B, is obtained from Life Technologies (Gaithersburg, MD); DH10B cells contain a mutation in the RECA gene (recA).

Systems, similar extracts from plants can be prepared as disclosed elsewhere in this application and used as disclosed in this example. We prepare cell-free extracts from HUH7 cells or other mammalian cells, as follows. We employ this protocol with essentially any mammalian cell including, for example, H1299 cells (human epithelial carcinoma, non-small cell lung cancer), C127I (immortal murine mammary epithelial cells), MEF (mouse embryonic fibroblasts), HEC-1-A (human uterine carcinoma), HCT15 (human colon cancer), HCT116 (human colon carcinoma), LoVo (human colon adenocarcinoma), and HeLa (human cervical carcinoma). We harvest approximately 2x108 cells. We then wash the cells immediately in cold hypotonic buffer (20 mM HEPES, pH7.5; 5 mM KCl; 1.5 mM MgCl<sub>2</sub>; 1 mM DTT) with 250 mM sucrose. We then resuspend the cells in cold hypotonic buffer without sucrose and after 15 minutes we lyse the cells with 25 strokes of a Dounce homogenizer using a tight fitting pestle. We incubate the lysed cells for 60 minutes on ice and centrifuge the sample for 15 minutes at 12000xg. The cytoplasmic fraction is enriched with nuclear proteins due to the extended co-incubation of the fractions following cell breakage.

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We then immediately aliquote and freeze the supernatant at -80°C. We determine the protein concentration in the extract by the Bradford assay.

We also perform these experiments with cell-free extracts obtained from fungal cells, including, for example, *S. cerevisiae* (yeast), *Ustilago maydis*, and *Candida albicans*. For example, we grow yeast cells into log phase in 2L YPD medium for 3 days at 30°C. We then centrifuge the cultures at 5000xg, resuspend the pellets in a 10% sucrose, 50 mM Tris, 1mM EDTA lysis solution and freeze them on dry ice. After thawing, we add KCl, spermidine and lyticase to final concentrations of 0.25 mM, 5 mM and 0.1 mg/ml, respectively. We incubate the suspension on ice for 60 minutes, add PMSF and Triton X100 to final concentrations of 0.1 mM and 0.1% and continue to incubate on ice for 20 minutes. We centrifuge the lysate at 3000xg for 10 minutes to remove larger debris. We then remove the supernatant and clarify it by centrifuging at 30000xg for 15 minutes. We then add glycerol to the clarified extract to a concentration of 10% (v/v) and freeze aliquots at -80°C. We determine the protein concentration of the extract by the Bradford assay.

Reaction mixtures of 50 µl are used, consisting of 10-30 µg protein of cell-free extract, which can be optionally substituted with purified proteins or enriched fractions, about 1.5 µg chimeric double-hairpin oligonucleotide or 0.55 µg single-stranded molecule (3S/25G or 6S/25G, see Figure 1). and 1 µg of plasmid DNA (see Figures 2 and 3) in a reaction buffer of 20 mM Tris, pH 7.4, 15 mM MgCl<sub>2</sub>, 0.4 mM DTT, and 1.0 mM ATP. Reactions are initiated with extract and incubated at 30°C for 45 min. The reaction is stopped by placing the tubes on ice and then immediately deproteinized by two phenol/chloroform (1:1) extractions. Samples are then ethanol precipitated. The nucleic acid is pelleted at 15,000 r.p.m. at 4°C for 30 min., is washed with 70% ethanol, resuspended in 50 µl H<sub>2</sub>0, and is stored at -20°C. 5 µl of plasmid from the resuspension (~100 ng) was transfected in 20 µl of DH10B cells by electroporation (400 V, 300  $\mu\text{F}$ , 4  $k\Omega$ ) in a Cell-Porator apparatus (Life Technologies). After electroporation, cells are transferred to a 14 ml Falcon snap-cap tube with 2 ml SOC and shaken at 37°C for 1 h. Enhancement of final kan colony counts is achieved by then adding 3 ml SOC with 10 µg/ml kanamycin and the cell suspension is shaken for a further 2 h at 37°C. Cells are then spun down at 3750 x g and the pellet is resuspended in 500 µl SOC. 200 µl is added undiluted to each of two kanamycin (50 µg/ml) agar plates and 200 μl of a 10<sup>5</sup> dilution is added to an ampicillin (100 μg/ml) plate. After overnight 37°C incubation, bacterial colonies are counted using an Accucount 1000 (Biologics). Gene conversion effectiveness is measured as the ratio of the average of the kan colonies on both plates per amp colonies multiplied by 10<sup>-5</sup> to correct for the amp dilution.

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The following procedure can also be used. 5 µl of resuspended reaction mixtures (total volume 50 µl) are used to transform 20 µl aliquots of electro-competent DH10B bacteria using a Cell-Porator apparatus (Life Technologies). The mixtures are allowed to recover in 1 ml SOC at 37°C for 1 hour at which time 50 µg/ml kanamycin or 12 µg/ml tetracycline is added for an additional 3 hours. Prior to plating, the bacteria are pelleted and resuspended in 200 µ1 of SOC. 100 µl aliquots are plated onto kan or tet agair plates and 100 µl of a 10<sup>-4</sup> dilution of the cultures are concurrently plated on agair plates containing 100 µg/ml of ampicillin. Plating is performed in triplicate using sterile Pyrex beads. Colony counts are determined by an Accu-count 1000 plate reader (Biologics). Each plate contains 200-500 ampicillin resistant colonies or 0-500 tetracycline or kanamycin resistant colonies. Resistant colonies are selected for plasmid extraction and DNA sequencing using an ABI Prism kit on an ABI 310 capillary sequencer (PE Biosystems).

Chimeric single-stranded oligonucleotides. In Figure 1 the upper strands of chimeric oligonucleotides I and II are separated into pathways resulting in the generation of single-stranded oligonucleotides that contain (Figure 1A) 2'-O-methyl RNA nucleotides or (Figure 1B) phosphorothioate linkages. Fold changes in repair activity for correction of kan<sup>s</sup> in the HUH7 cell-free extract are presented in parenthesis. Each single-stranded oligonucleotide is 25 bases in length and contains a G residue mismatched to the complementary sequence of the kan<sup>s</sup> gene.

Molecules bearing 3, 6, 8, 10 and 12 phosphorothioate linkages in the terminal regions at each end of a backbone with a total of 24 linkages (25 bases) are tested in the kan<sup>s</sup> system. Alternatively, molecules bearing 2, 4, 5, 7, 9 and 11 in the terminal regions at each end are tested. The results of one such experiment, presented in Table 1 and Figure 1B, illustrate an enhancement of correction activity directed by some of these modified structures. In this illustrative example, the most efficient molecules contained 3 or 6 phosphorothioate linkages at each end of the 25-mer; the activities are approximately equal (molecules IX and X with results of 3.09 and 3.7 respectively). A reduction in alteration activity may be observed as the number of modified linkages in the molecule is further increased. Interestingly, a single-strand molecule containing 24 phosphorothioate linkages is minimally active suggesting that this backbone modification when used throughout the molecule supports only a low level of targeted gene repair or alteration. Such a non-altering, completely modified molecule can provide a baseline control for determining efficiency of correction for a specific oligonucleotide molecule of known sequence in defining the optimum oligonucleotide for a particular alteration event.

The efficiency of gene repair directed by phosphorothioate-modified, single-stranded molecules, in a length dependent fashion, led us to examine the length of the RNA modification used in

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the original chimera as it relates to correction. Construct III represents the "RNA-containing" strand of chimera I and, as shown in Table 1 and Figure 2A, it promotes inefficient gene repair. But, as shown in the same figure, reducing the RNA residues on each end from 10 to 3 increases the frequency of repair. At equal levels of modification, however, 25-mers with 2'-O-methyl ribonucleotides were less effective gene repair agents than the same oligomers with phosphorothicate linkages. These results reinforce the fact that an RNA containing oligonucleotide is not as effective in promoting gene repair or alteration as a modified DNA oligonucleotide.

Repair of the kanamycin mutation requires a G→C exchange. To confirm that the specific desired correction alteration was obtained, colonies selected at random from multiple experiments are processed and the isolated plasmid DNA is sequenced. As seen in Figure 4, colonies generated through the action of the single-stranded molecules 3S/25G (IX), 6S/25G (X) and 8S/25G (XI) respectively contained plasmid molecules harboring the targeted base correction. While a few colonies appeared on plates derived from reaction mixtures containing 25-mers with 10 or 12 thioate linkages on both ends, the sequences of the plasmid molecules from these colonies contain nonspecific base changes. In these illustrative examples, the second base of the codon is changed (see Figure 3). These results show that modified single-strands can direct gene repair, but that efficiency and specificity are reduced when the 25-mers contain 10 or more phosphorothioate linkages at each end.

In Figure 1, the numbers 3, 6, 8, 10, 12 and 12.5 respectively indicate how many phosphorothioate linkages (S) or 2'-O-methyl RNA nucleotides (R) are at each end of the examplified molecule although other molecules with 2, 4, 5, 7, 9 and 11 modifications at each end can also be tested. Hence oligo 12S/25G represents a 25-mer oligonucleotide which contains 12 phosphorothioate linkages on each side of the central G target mismatch base producing a fully phosphorothioate linked backbone, displayed as a dotted line. The dots are merely representative of a linkage in the figure and do not depict the actual number of linkages of the oligonucleotide. Smooth lines indicate DNA residues, wavy lines indicate 2'-O-methyl RNA residues and the carat indicates the mismatched base site (G).

Correction of a mutant kanamycin gene in cultured mammalian cells. Although this portion of this example is directed to cultured mammalian cells, comparable methods may be used using cultured plant cells or protoplasts of those cells from the plant species disclosed herein. The experiments are performed using different eukaryotic cells including plant and mammalian cells, including, for example, 293 cells (transformed human primary kidney cells), HeLa cells (human cervical carcinoma), and H1299 (human epithelial carcinoma, non-small cell lung cancer). HeLa cells are grown at 37°C and 5% CO<sub>2</sub> in a humidified incubator to a density of 2 x 10<sup>s</sup> cells/ml in an 8 chamber slide (Lab-Tek). After replacing the

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regular DMEM with Optimem, the cells are co-transfected with 10 µg of plasmid pAURNeo(-)FIAsH and  $5~\mu g$  of modified single-stranded oligonucleotide (3S/25G) that is previously complexed with 10  $\mu g$ lipofectamine, according to the manufacturer's directions (Life Technologies). The cells are treated with the liposome-DNA-oligo mix for 6 hrs at 37°C. Treated cells are washed with PBS and fresh DMEM is added. After a 16-18 hr recovery period, the culture is assayed for gene repair. The same oligonucleotide used in the cell-free extract experiments is used to target transfected plasmid bearing the kan<sup>s</sup> gene. Correction of the point mutation in this gene eliminates a stop codon and restores full expression. This expression can be detected by adding a small non-fluorescent ligand that bound to a C-C-R-E-C-C sequence in the genetically modified carboxy terminus of the kan protein, to produce a highly fluorescent complex (FIAsH system, Aurora Biosciences Corporation). Following a 60 min incubation at room temperature with the ligand (FIAsH-EDT2), cells expressing full length kan product acquire an intense green fluorescence detectable by fluorescence microscopy using a fluorescein filter set. Similar experiments are performed using the HygeGFP target as described in Example 2 with a variety of mammalian cells, including, for example, COS-1 and COS-7 cells (African green monkey), and CHO-K1 cells (Chinese hamster ovary). The experiments are also performed with PG12 cells (rat pheochromocytoma) and ES cells (human embryonic stem cells).

Summary of experimental results. Tables 1, 2 and 3 respectively provide data on the efficiency of gene repair directed by single-stranded oligonucleotides. Table 1 presents data using a cell-free extract from human liver cells (HUH7) to catalyze repair of the point mutation in plasmid pkan<sup>5</sup>m4021 (see Figure 1). Table 2 illustrates that the oligomers are not dependent on MSH2 or MSH3 for optimal gene repair activity. Table 3 illustrates data from the repair of a frameshift mutation (Figure 3) in the tet gene contained in plasmid pTet∆208. Table 4 illustrates data from repair of the pkan<sup>5</sup>m4021 point mutation catalyzed by plant cell extracts prepared from canola and musa (banana). Colony numbers are presented as kan¹ or tet¹ and fold increases (single strand versus double hairpin) are presented for kan¹ in Table 1.

Figure 5A is a confocal picture of HeLa cells expressing the corrected fusion protein from an episomal target. Gene repair is accomplished by the action of a modified single-stranded oligonucleotide containing 3 phosphorothicate linkages at each end (3S/25G). Figure 5B represents a "Z-series" of HeLa cells bearing the corrected fusion gene. This series sections the cells from bottom to top and illustrates that the fluorescent signal is "inside the cells".

Results. In summary, we have designed a novel class of single-stranded oligonucleotides with backbone modifications at the termini and demonstrate gene repair/conversion

activity in mammalian and plant cell-free extracts. We confirm that the all DNA strand of the RNA-DNA double-stranded double hairpin chimera is the active component in the process of gene repair. In some cases, the relative frequency of repair by the novel oligonucleotides of the invention is elevated approximately 3-4-fold in certain embodiments when compared to frequencies directed by chimeric RNA-DNA double hairpin oligonucleotides.

This strategy centers around the use of extracts from various sources to correct a mutation in a plasmid using a modified single-stranded or a chimeric RNA-DNA double hairpin oligonucleotide. A mutation is placed inside the coding region of a gene conferring antibiotic resistance in bacteria, here kanamycin or tetracycline. The appearance of resistance is measured by genetic readout in *E.coli* grown in the presence of the specified antibiotic. The importance of this system is that both phenotypic alteration and genetic inheritance can be measured. Plasmid pKsm4021 contains a mutation (T→G) at residue 4021 rendering it unable to confer antibiotic resistance in *E.coli*. This point mutation is targeted for repair by oligonucleotides designed to restore kanamycin resistance. To avoid concerns of plasmid contamination skewing the colony counts, the directed correction is from G→C rather than G→T (wild-type). After isolation, the plasmid is electroporated into the DH10B strain of *E.coli*, which contains inactive RecA protein. The number of kanamycin colonies is counted and normalized by ascertaining the number of ampicillin colonies, a process that controls for the influence of electroporation. The number of colonies generated from three to five independent reactions was averaged and is presented for each experiment. A fold increase number is recorded to aid in comparison.

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The original RNA-DNA double hairpin chimera design, e.g., as disclosed in U.S. Patent 5,565,350, consists of two hybridized regions of a single-stranded oligonucleotide folded into a double hairpin configuration. The double-stranded targeting region is made up of a 5 base pair DNA/DNA segment bracketed by 10 base pair RNA/DNA segments. The central base pair is mismatched to the corresponding base pair in the target gene. When a molecule of this design is used to correct the kan<sup>s</sup> mutation, gene repair is observed (I in Figure 1A). Chimera II (Figure 1B) differs partly from chimera I in that only the DNA strand of the double hairpin is mismatched to the target sequence. When this chimera was used to correct the kan<sup>s</sup> mutation, it was twice as active. In the same study, repair function could be further increased by making the targeting region of the chimera a continuous RNA/DNA hybrid.

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Frame shift mutations are repaired. By using plasmid pT\*\Delta 208, described in Figure 1(C) and Figure 3, the capacity of the modified single-stranded molecules that showed activity in correcting a point mutation, can be tested for repair of a frameshift. To determine efficiency of correction of the mutation, a chimeric oligonucleotide (Tet I), which is designed to insert a T residue at position 208, is

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used. A modified single-stranded oligonucleotide (Tet IX) directs the insertion of a T residue at this same site. Figure 3 illustrates the plasmid and target bases designated for change in the experiments. When all reaction components are present (extract, plasmid, oligomer), tetracycline resistant colonies appear. The colony count increases with the amount of oligonucleotide used up to a point beyond which the count falls off (Table 3). No colonies above background are observed in the absence of either extract or oligonucleotide, nor when a modified single-stranded molecule bearing perfect complementarity is used. Figure 3 represents the sequence surrounding the target site and shows that a T residue is inserted at the correct site. We have isolated plasmids from fifteen colonies obtained in three independent experiments and each analyzed sequence revealed the same precise nucleotide insertion. These data suggest that the single-stranded molecules used initially for point mutation correction can also repair nucleotide deletions.

Comparison of phosphorothioate oligonucleotides to 2'-O-methyl substituted oligonucleotides. From a comparison of molecules VII and XI, it is apparent that gene repair is more subject to inhibition by RNA residues than by phosphorothioate linkages. Thus, even though both of these oligonucleotides contain an equal number of modifications to impart nuclease resistance, XI (with 16 phosphorothioate linkages) has good gene repair activity while VII (with 16 2'-O-methyl RNA residues) is inactive. Hence, the original chimeric double hairpin oligonucleotide enabled correction directed, in large part, by the strand containing a large region of contiguous DNA residues.

Oligonucleotides can target multiple nucleotide alterations within the same template. The ability of individual single-stranded oligonucleotides to correct multiple mutations in a single target template is tested using the plasmid pK\*m4021 and the following single-stranded oligonucleotides modified with 3 phosphorothioate linkages at each end (indicated as underlined nucleotides): Oligo1 is a 25-mer with the sequence TTCGATAAGCCTATGCTGACCCGTG corrects the original mutation present in the kanamycin resistance gene of pK\*m4021 as well as directing another alteration 2 basepairs away in the target sequence (both indicated in boldface); Oligo2 is a 70-mer with the 5'-end sequence TTCGGCTACGACTGGGCACAACAGACAATTGGC with the remaining nucleotides being completely complementary to the kanamycin resistance gene and also ending in 3 phosphorothioate linkages at the 3' end. Oligo2 directs correction of the mutation in pK\*m4021 as well as directing another alteration 21 basepairs away in the target sequence (both indicated in boldface).

We also use additional oligonucleotides to assay the ability of individual oligonucleotides to correct multiple mutations in the pK\*M4021 plasmid. These include, for example, a second 25-mer that alters two nucleotides that are three nucleotides apart with the sequence 5'-

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TTGTGCCCAGTCGTATCCGAATAGC-3'; a 70-mer that alters two nucleotides that are 21 nucleotides apart with the sequence 5'-CATCAGAGCAGCCAATTGTCTGTTGTGCCCAGTCGTAGCCGAA TAGCCTCTCCACCCAAGCGGCCGGAGA-3'; and another 70-mer that alters two nucleotides that are 21 nucleotides apart with the sequence 5'-

GCTGACAGCCGGAACACGGCGGCATCAGAGCAGCCAATTGTCTGTTGTCCCAGTCGTAGCCGAAT AGCCT-3'. The nucleotides in the oligonucleotides that direct alteration of the target sequence are underlined and in boldface. These oligonucleotides are modified in the same way as the other oligonucleotides of the invention.

We assay correction of the original mutation in pKsm4021 by monitoring kanamycin resistance (the second alterations which are directed by Oligo2 and Oligo3 are silent with respect to the kanamycin resistance phenotype). In addition, in experiments with Oligo2, we also monitor cleavage of the resulting plasmids using the restriction enzyme Tsp509l which cuts at a specific site present only when the second alteration has occurred (at ATT in Oligo2). We then sequence these clones to determine whether the additional, silent alteration has also been introduced. The results of an analysis are presented below:

	Oligo1 (25-mer)	Oligo2 (70-mer)
Clones with both sites changed	9	7
Clones with a single site changed	0	2
Clones that were not changed	4	1

Nuclease sensitivity of unmodified DNA oligonucleotide. Electrophoretic analysis of nucleic acid recovered from the cell-free extract reactions conducted here confirm that the unmodified single-stranded 25-mer did not survive incubation whereas greater than 90% of the terminally modified oligos did survive (as judged by photo-image analyses of agarose gels).

Plant extracts direct repair. The modified single-stranded constructs can be tested in plant cell extracts. We have observed gene alteration using extracts from multiple plant sources, including, for example, Arabidopsis, tobacco, banana, maize, soybean, canola, wheat, spinach as well as spinach chloroplast extract or extracts made from other plant cells disclosed herein. We prepare the extracts by grinding plant tissue or cultured cells under liquid nitrogen with a mortar and pestle. We extract 3 ml of the ground plant tissue with 1.5 ml of extraction buffer (20 mM HEPES, pH7.5; 5 mM KCl;

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1.5 mM MgCl<sub>2</sub>; 10 mM DTT; and 10% [v/v] glycerol). Some plant cell-free extracts also include about 1% (w/v) PVP. We then homogenize the samples with 15 strokes of a Dounce homogenizer. Following homogenization, we incubate the samples on ice for 1 hour and centrifuge at 3000 x g for 5 minutes to remove plant cell debris. We then determine the protein concentration in the supernatants (extracts) by Bradford assay. We dispense 100  $\mu$ g (protein) aliquots of the extracts which we freeze in a dry ice-ethanol bath and store at -80°C.

We describe experiments using two sources here: a dicot (canola) and a monocot (banana, *Musa acuminata* cv. Rasthali). Each vector directs gene repair of the kanamycin mutation (Table 4); however, the level of correction is elevated 2-3 fold relative to the frequency observed with the chimeric oligonucleotide. These results are similar to those observed in the mammalian system wherein a significant improvement in gene repair occurred when modified single-stranded molecules were used.

Tables are attached hereto.

Table I

Gene repair activity is directed by single-stranded oligonucleotides.

			•	
Oligonucleotide	Plasmid	Extract (ug)	kan' colonies	Fold in annual
I	pK <sup>S</sup> m4021	10	300	Fold increase
I	1	20	418	1.0
П		10	537	1.0x
П		20	748	1.50
Ш	j	10	3	1.78x
Ш	ı	20		
IV		10	5	0.01x
IV		20	112	•
V	ı		96	0.22x
V	į.	10	217	
VI	ł	20	342	0.81x
VI	į	10	6	
VII		20	39	0.093x
VΪ	ĺ	10	0	
VIII	į .	20	0	0x
VIII		10 .	3	
IX		20	5	0.01x
IX	1	10	936	
X	'	20	1295	3.09x
x	1	10	1140	0.002
	ł	20	1588	3.7x
XI	- 1	10 .	480	
XI	1	20	681	1.6x
XII		10	18	1.04
XII	1	20	25	0.059x
XIII		10	Ō	U.UJ7A
XIII		20	4	0.000
• •	1	20	Ŏ	0.009 <sub>X</sub>
I	▼	•	ŏ	•
			V	

Plasmid pK<sup>4</sup>m4021 (1μg), the indicated oligonucleotide (1.5 μg chimeric oligonucleotide or 0.55 μg single-stranded oligonucleotide; molar ratio of oligo to plasmid of 360 to 1) and either 10 or 20 μg of HUH7 cell-free extract were incubated 45 min at 37°C. Isolated plasmid DNA was electroporated into *E. coli* (strain DH10B) and the number of kan<sup>7</sup> colonies counted. The data represent the number of kanamycin resistant colonies per 10<sup>6</sup> ampicillin resistant colonies generated from the same reaction and is the average of three

experiments (standard deviation usually less than +/- 15%). Fold increase is defined relative to 418 kan' colonies (second reaction) and in all reactions was calculated using the 20µg sample.

Table II

Modified single-stranded oligomers are not dependent on MSH2 or MSH3 for optimal gene repair activity.

A. Oligonucleotide	Plasmid	Extract	kan <sup>r</sup> colonies
IX (3\$/25G)		HUH7	637
X (6S/25G)		HUH7	836
IX ·	1	MEF2 <sup>→</sup>	781
X	ļ	MEF2	676
IX	İ	MEF3	582
X	İ	MEF3	<i>5</i> 30
. <b>IX</b>	- 1	MEF**	. 332
X	ł	MEF**	497
•	.	MEF2	10
•	[	MEF3	5
•	₩	MEF	14

Chimeric oligonucleotide (1.5 µg) or modified single-stranded oligonucleotide (0.55 µg) was incubated with 1µg of plasmid pK m4021 and 20µg of the indicated extracts. MEF represents mouse embryonic fibroblasts with either MSH2 (2<sup>+/-</sup>) or MSH3 (3<sup>+/-</sup>) deleted. MEF indicates wild-type mouse embryonic fibroblasts. The other reaction components were then added and processed through the bacterial readout system. The data represent the number of kanamycin resistant colonies per 10<sup>6</sup> ampicillin resistant colonies.

Table III

Frameshift mutation repair is directed by single-stranded oligonucleotides

Oligonucleotide	Plasmid	Extract	tet colonies
Tet IX (3S/25A; 0.5 μg)	pT Δ208 (1μg)		- 0
•		20µg	0
Tet IX (0.5 μg)		1	48 .
Tet IX (1.5 μg)		1	130
Tet IX (2.0 μg)	'	ł	68
Tet I (chimera; 1.5 μg)	▼	\	48

Each reaction mixture contained the indicated amounts of plasmid and oligonucleotide. The extract used for these experiments came from HUH7 cells. The data represent the number of tetracycline resistant colonies per  $10^6$  ampicillin resistant colonies generated from the same reaction and is the average of 3 independent experiments. Tet I is a chimeric oligonucleotide and Tet IX is a modified single-stranded oligonucleotide that are designed to insert a T residue at position 208 of pT $^4\Delta208$ . These oligonucleotides are equivalent to structures I and IX in Figure 2.

Table IV

Plant cell-free extracts support gene repair by single-stranded oligonucleotides

<u>Oligonucleotide</u>	Plasmid	Extract	kan' colonies
II (chimera)	pK <sup>8</sup> m4021	30µg Canola	337
IX (3S/25G)	1	Canola	763
X (6S/25G)		Canola	882
П		Musa	203
IX		Musa	. 343
X		Musa	746
•		Canola	0
•		Musa	0
IX		- Canola	0
X	. ↓	- Musa	0

Canola or Musa cell-free extracts were tested for gene repair activity on the kanamycin-sensitive gene as previously described in (18). Chimeric oligonucleotide II (1.5 µg) and modified single-stranded oligonucleotides IX and X (0.55µg) were used to correct pK<sup>S</sup>th4021. Total number of kan<sup>r</sup> colonies are present per 10<sup>7</sup> ampicillin resistant colonies and represent an average of four independent experiments.

Table V

Gene repair activity in cell-free extracts prepared from yeast (Saccharomyces cerevisiae)

				900 - 8
Cell-type	Plasmid	Chimeric Ulgo	22 0489	SS CHES KEN / AMP & LV
Wild type	pKan'm4021	1µg		0.36
Wild type			<u>왕</u>	10.72
ARAD52			ant .	17.41
APMS1 APMS1	<b>→</b>	<b>9</b>	148	3.23

In this experiment, the kan' gene in pKan' 4021 is corrected by either a chimeric double-hairpin oligonucleotide or a single-stranded oligonucleotide comaining three thioate linkages at each end (3S/25G).

# EXAMPLE 2 Yeast Cell Targeting Assay Method for Base Alteration and Preferred Oligonucleotide Selection

In this example, single-stranded oligonucleotides with modified backbones and double-hairpin oligonucleotides with chimeric, RNA-DNA backbones are used to measure gene repair using two episomal targets with a fusion between a hygromycin resistance gene and eGFP as a target for gene repair. These plasmids are pAURHYG(rep)GFP, which contains a point mutation in the hygromycin resistance gene (Figure 7), pAURHYG(ins)GFP, which contains a single-base insertion in the hygromycin resistance gene (Figure 7) and pAURHYG(Δ)GFP which has a single base deletion. We also use the plasmid containing a wild-type copy of the hygromycin-eGFP fusion gene, designated pAURHYG(wt)GFP, as a control. These plasmids also contain an aureobasidinA resistance gene. In pAURHYG(rep)GFP, hygromycin resistance gene function and green fluorescence from the eGFP protein are restored when a G at position 137, at codon 46 of the hygromycin B coding sequence, is converted to a C thus removing a premature stop codon in the hygromycin resistance gene coding region. In pAURHYG(ins)GFP, hygromycin resistance gene function and green fluorescence from the eGFP protein are restored when an A inserted between nucleotide positions 136 and 137, at codon 46 of the hygromycin B coding sequence, is deleted and a C is substituted for the T at position 137, thus correcting a frameshift mutation and restoring the reading frame of the hygromycin-eGFP fusion gene.

We synthesize the set of three yeast expression constructs pAURHYG(rep)eGFP, pAURHYG(∆)eGFP, pAURHYG(ins)eGFP, that contain a point mutation at nucleotide 137 of the hygromycin-B coding sequence as follows. (rep) indicates a T137→G replacement, (∆) represents a deletion of the G137 and (ins) represents an A insertion between nucleotides 136 and 137. We construct this set of plasmids by excising the respective expression cassettes by restriction digest from pHyg(x)EGFP and ligation into pAUR123 (Panvera, CA). We digest 10 µg pAUR123 vector DNA, as well as, 10 µg of each pHyg(x)EGFP construct with Kpnl and Sall (NEB). We gel purify each of the DNA fragments and prepare them for enzymatic ligation. We ligate each mutated insert into pHygEGFP vector at 3:1 molar ratio using T4 DNA ligase (Roche). We screen clones by restriction digest, confirm by Sanger dideoxy chain termination sequencing and purify using a Qiagen maxiprep kit.

We use this system to assay the ability of five oligonucleotides (shown in Figure 8) to support correction under a variety of conditions. The oligonucleotides which direct correction of the mutation in pAURHYG(rep)GFP can also direct correction of the mutation in pAURHYG(ins)GFP. Three of the four oligonucleotides (HygE3T/25, HygE3T/74 and HygGG/Rev) share the same 25-base sequence surrounding the base targeted for alteration. HygGG/Rev is an RNA-DNA chimeric double hairpin

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oligonucleotide of the type described in the prior art. One of these oligonucleotides, HygE3T/74, is a 74-base oligonucleotide with the 25-base sequence centrally positioned. The fourth oligonucleotide, designated HygE3T/74α, is the reverse complement of HygE3T/74. The fifth oligonucleotide, designated Kan70T, is a non-specific, control oligonucleotide which is not complementary to the target sequence. Alternatively, an oligonucleotide of identical sequence but lacking a mismatch to the target or a completely thioate modified oligonucleotide or a completely 2-0-methylated modified oligonucleotide may be used as a control. Alternatively, oligonucleotides containing one, two, three, four, five, six, eight, ten or more LNA modifications on at least one of the two termini (and preferrably the 3' terminus) may be used in different embodiments.

Oligonucleotide synthesis and cells. We synthesized and purified the chimeric, double-

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hairpin oligonucleotides and single-stranded oligonucleotides (including those with the indicated modifications) as described in Example 1. Plasmids used for assay were maintained stably in yeast (Saccharomyces cerevisiae) strain LSY678 MAT at low copy number under aureobasidin selection. Plasmids and oligonucleotides are introduced into yeast cells by electroporation as follows: to prepare electrocompetent yeast cells, we inoculate 10 ml of YPD media from a single colony and grow the cultures overnight with shaking at 300 rpm at 30°C. We then add 30 ml of fresh YPD media to the overnight cultures and continue shaking at 30°C until the  $OD_{soo}$  was between 0.5 and 1.0 (3-5 hours). We then wash the cells by centrifuging at 4°C at 3000 rpm for 5 minutes and twice resuspending the cells in 25 ml ice-cold distilled water. We then centrifuge at 4°C at 3000 rpm for 5 minutes and resuspend in 1 ml ice-cold 1M sorbitol and then finally centrifuge the cells at 4°C at 5000 rpm for 5 minutes and resuspend the cells in 120 µl 1M sorbitol. To transform electrocompetent cells with plasmids or oligonucleotides, we mix 40 µl of cells with 5 µg of nucleic acid, unless otherwise stated, and incubate on ice for 5 minutes. We then transfer the mixture to a 0.2 cm electroporation cuvette and electroporate with a BIO-RAD Gene Pulser apparatus at 1.5 kV, 25  $\mu$ F, 200  $\Omega$  for one five-second pulse. We then immediately resuspend the cells in 1 ml YPD supplemented with 1M sorbitol and incubate the cultures at 30°C with shaking at 300 rpm for 6 hours. We then spread 200 µl of this culture on selective plates containing 300 µg/ml hygromycin and spread 200 µl of a 10<sup>5</sup> dilution of this culture on selective plates containing 500 ng/ml aureobasidinA and/or and incubate at 30°C for 3 days to allow individual yeast colonies to grow. We then count the colonies on the plates and calculate the gene conversion efficiency by determining the number of hygromycin resistance colonies per 10<sup>5</sup> aureobasidinA resistant colonies.

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Frameshift mutations are repaired in yeast cells. We test the ability of the oligonucleotides shown in Figure 8 to correct a frameshift mutation in vivo using LSY678 yeast cells

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containing the plasmid pAURHYG(ins)GFP. These experiments, presented in Table 6, indicate that these oligonucleotides can support gene correction in yeast cells. These data reinforce the results described in Example 1 indicating that oligonucleotides comprising phosphorothioate linkages facilitate gene correction much more efficiently than control duplex, chimeric RNA-DNA oligonucleotides. This gene correction activity is also specific as transformation of cells with the control oligonucleotide Kan70T produced no hygromycin resistant colonies above background and thus Kan70T did not support gene correction in this system. In addition, we observe that the 74-base oligonucleotide (HygE3T/74) corrects the mutation in pAURHYG(ins)GFP approximately five-fold more efficiently than the 25-base oligonucleotide (HygE3T/25). We also perform control experiments with LSY678 yeast cells containing the plasmid pAURHYG(wt)GFP. With this strain we observed that even without added oligonucleotides, there are too many hygromycin resistant colonies to count.

We also use additional oligonucleotides to assay the ability of individual oligonucleotides to correct multiple mutations in the pAURHYG(x)eGFP plasmid. These include, for example, one that alters two basepairs that are 3 nucleotides apart is a 74-mer with the sequence 5'CTCGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGGTACGTCCTGCGGGTAAATAGCTGCGCCGATG
GTTTCTAC-3'; a 74-mer that alters two basepairs that are 15 nucleotides apart with the sequence 5'CTCGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGATACGTCCTGCGGGTAAACAGCTGCGCCGATG
GTTTCTAC-3'; and a 74-mer that alters two basepairs that are 27 nucleotides apart with the sequence 5'CTCGTGCTTTCAGCTTCGATGTAGGAGGGCGTGGATACGTCCTGCGGGTAAATAGCTGCGCCGACG
GTTTCTAC. The nucleotides in these oligonucleotides that direct alteration of the target sequence are underlined and in boldface. These oligonucleotides are modified in the same ways as the other oligonucleotides of the invention.

Oligonucleotides targeting the sense strand direct gene correction more efficiently. We compare the ability of single-stranded oligonucleotides to target each of the two strands of the target sequence of both pAURHYG(ins)GFP and pAURHYG(rep)GFP. These experiments, presented in Tables 7 and 8, indicate that an oligonucleotide, HygE3T/74 $\alpha$ , with sequence complementary to the sense strand (i.e. the strand of the target sequence that is identical to the mRNA) of the target sequence facilitates gene correction approximately ten-fold more efficiently than an oligonucleotide, HygE3T/74, with sequence complementary to the non-transcribed strand which serves as the template for the synthesis of RNA. As indicated in Table 7, this effect was observed over a range of oligonucleotide concentrations from 0-3.6  $\mu$ g, although we did observe some variability in the difference between the two oligonucleotides (indicated in Table 7 as a fold difference between HygE3T/74 $\alpha$  and HygE3T/74).

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Furthermore, as shown in Table 8, we observe increased efficiency of correction by HygE3T/74 $\alpha$  relative to HygE3T/74 regardless of whether the oligonucleotides were used to correct the base substitution mutation in pAURHYG(rep)GFP or the insertion mutation in pAURHYG(ins)GFP. The data presented in Table 8 further indicate that the single-stranded oligonucleotides correct a base substitution mutation more efficiently than an insertion mutation. However, this last effect was much less pronounced and the oligonucleotides of the invention are clearly able efficiently to correct both types of mutations in yeast cells. In addition, the role of transcription is investigated using plasmids with inducible promoters such as that described in Figure 10.

Optimization of oligonucleotide concentration. To determine the optimal concentration of oligonucleotide for the purpose of gene alteration, we test the ability of increasing concentrations of Hyg3T/74 $\alpha$  to correct the mutation in pAURHYG(rep)GFP contained in yeast LSY678. We chose this assay system because our previous experiments indicated that it supports the highest level of correction. However, this same approach could be used to determine the optimal concentration of any given oligonucleotide. We test the ability of Hyg3T/74 $\alpha$  to correct the mutation in pAURHYG(rep)GFP contained in yeast LSY678 over a range of oligonucleotide concentrations from 0-10.0  $\mu$ g. As shown in Table 9, we observe that the correction efficiency initially increases with increasing oligonucleotide concentration, but then declines at the highest concentration tested.

Tables are attached hereto.

Table 6

Correction of an insertion mutation in pAURHYG(ins)GFP by HygGG/Rev, HygE3T/25 and HygE3T/74

Oligonucleotide Tested	Colonies on Hygromycin	Colonies on Aureobasidin (/10 <sup>5</sup> )	Correction Efficiency
HygGG/Rev	3	157	0.02
HygE3T/25	64	147	0.44
HygE3T/74	280	174	1.61
Kan70T	0	_	_

Table 7

An oligonucleotide targeting the sense strand of the target sequence corrects more efficiently.

Amount of Oligonucleotide (µg)	Colonies per hy	ygromycin plate
·	HygE3T/74	HygE3T/74α
0	0	0
0.6	24	128 (8.4x)*
1.2	69	140 (7.5x)*
2.4	62	167 (3.8x)*
3.6	29	367 (15x)*

<sup>\*</sup> The numbers in parentheses represent the fold increase in efficiency for targeting the non-transcribed strand as compared to the other strand of a DNA duplex that encodes a protein.

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Table 8

Correction of a base substitution mutation is more efficient than correction of a frame shift mutation.

Oligonucleotide Tested (5 µg)	Plasmid tested (co	ontained in LSY678)
	pAURHYG(ins)GFP	pAURHYG(rep)GFP
HygE3T/74	72	277
HygE3T/74α	1464	2248
Kan70T	0	0

Table 9

Optimization of oligonucleotide concentration in electroporated yeast cells.

Amount (µg)	Colonies on hygromycin	Colonies on aureobasidin (/10 <sup>5</sup> )	Correction efficiency
0	0	67	0
1.0	5	64	0.08
2.5	47	30	1.57
5.0	199	33	6.08
7.5	383	39	9.79
10.0	191	33	5.79

### Example 3 Cultured Cell Manipulation

Although disclosure in this example is directed to use of stem cells or human blood cells and microinjection, the microinjection procedures may also be used with cultured plant cells or protoplasts using any plant species, including those disclosed herein. Mononuclear cells are isolated from human umbilical cord blood of normal donors using Ficoll Hypaque (Pharmacia Biotech, Uppsala, Sweden) density centrifugation. CD34+ cells are immunomagnetically purified from mononuclear cells using either

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the progenitor or Multisort Kits (Miltenyi Biotec, Auburn, CA). Lin<sup>-</sup>CD38<sup>-</sup> cells are purified from the mononuclear cells using negative selection with StemSep system according to the manufacturer's protocol (Stem Cell Technologies, Vancouver, CA). Cells used for microinjection are either freshly isolated or cryopreserved and cultured in Stem Medium (S Medium) for 2 to 5 days prior to microinjection. S Medium contains Iscoves' Modified Dulbecco's Medium without phenol red (IMDM) with 100 µg/ml glutamine/penicillin/streptomycin, 50 mg/ml bovine serum albumin, 50 µg/ml bovine pancreatic insulin, 1 mg/ml human transferrin, and IMDM; Stem Cell Technologies), 40 µg/ml low-density lipoprotein (LDL; Sigma, St. Louis, MO), 50 mM HEPEs buffer and 50 µM 2-mercaptoethanol, 20 ng/ml each of thrombopoietin, fit-3 ligand, stem cell factor and human IL-6 (Pepro Tech Inc., Rocky Hill, NJ). After microinjection, cells are detached and transferred in bulk into wells of 48 well plates for culturing.

35 mm dishes are coated overnight at 4° C with 50 µg/ml Fibronectin (FN) fragment CH-296 (Retronectin; TaKaRa Biomedicals, Panvera, Madison, WI) in phosphate buffered saline and washed with IMDM containing glutamine/penicillin/streptomycin. 300 to 2000 cells are added to cloning rings and attached to the plates for 45 minutes at 37° C prior to microinjection. After incubation, cloning rings are removed and 2 ml of S Medium are added to each dish for microinjection. Pulled injection needles with a range of 0.22 µm to 0.3 µm outer tip diameter are used. Cells are visualized with a microscope equipped with a temperature controlled stage set at 37° C and injected using an electronically interfaced Eppendorf Micromanipulator and Transjector. Successfully injected cells are intact, alive and remain attached to the plate post injection. Molecules that are flourescently labeled allow determination of the amount of oligonucleotide delivered to the cells.

For in vitro erythropoiesis from Lin<sup>\*</sup>CD38<sup>\*</sup> cells, the procedure of Malik, 1998 can be used. Cells are cultured in ME Medium for 4 days and then cultured in E Medium for 3 weeks. Erythropoiesis is evident by glycophorin A expression as well as the presence of red color representing the presence of hemoglobin in the cultured cells. The injected cells are able to retain their proliferative capacity and the ability to generate myeloid and erythoid progeny. CD34+ cells can convert a normal A ( $\beta^A$ ) to sickle T ( $\beta^S$ ) mutation in the  $\beta$ -globin gene or can be altered using any of the oligonucleotides of the invention herein for correction or alteration of a normal gene to a mutant gene. Alternatively, stem cells can be isolated from blood of humans having genetic disease mutations and the oligonucleotides of the invention can be used to correct a defect or to modify genomes within those cells.

Alternatively, non-stem cell populations of cultured cells can be manipulated using any method known to those of skill in the art including, for example, the use of polycations, cationic lipids,

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liposomes, polyethylenimine (PEI), electroporation, biolistics, calcium phosphate precipitation, or any other method known in the art.

Biolistic delivery of oligonucleotide into plant cells may be accomplished according to the following method. One milliliter of packed cell volume of plant cell suspensions are subcultured onto plates containing solid medium [with Murashige and Skoog salts from Gibco/BRL, 500 mg/liter Mes, 1 ma/liter thiamin, 100 mg/liter myo-inositol, 180 mg/liter KH2PO4, 2.21 mg/liter 2,4-dichlorophenoxyacetic acid (2,4-D), and 30 g/liter sucrose (pH 5.7) and having 8 g/liter agar-agar from Sigma added before autoclaving). By using a helium-driven particle gun such as that from BioRad and following manufacturers directions, oligonucleotides may be introduced to cells after precipitation onto 1 micrometer or comparable gold microcarriers (Bio-Rad). To precipitate onto microcarriers, 35 microliters of a particle suspension (60 mg of microcarriers per ml of 100% ethanol) is transferred to a 1.5 ml microcentrifuge tube, which is agitated on a vortex mixer. Then 40 microliter of resuspended oligonucleotide (60 ng/microliter water) is added; then 75 microliter of ice-cold 2.5 M CaCl2 is added; then 75 microliter of icecold 0.1 M spermidine is added. The tube is mixed vigorously or a vortex mixer for 10 min at room temperature. The particles are allowed to settle for 10 min and are centrifuged at 11,750 g for 30 sec. The supernatant is removed and the particles are resuspended in 50 microliter of 100% ethanol. An aliquot of 10 microliter of the resuspended particles are applied to each macro-projectile which is used to bombard each plate once at 900 psi (1 psi = 6.89 kPa) with a gap distance (distance from power source to macroprojectile) of 1 cm and a target distance (distance from microprojectile launch site to target material) of 10 cm.

An alternative method of delivery can be used as follows. Cultured cells are suspended in liquid N6 medium and then plated on a VWR Scientific glass fiber filter. About 0.4 microgram of oligonucleotide are precipitated with 15 microliter of 2.5 mM CaCl2 and 5 microliter of 0.1 M spermidine onto 25 microgram of 1.0 micrometer gold particles. Microprojectile bombardment is performed by using a Bio-Rad PDS-1000 He particle delivery system or comparable machine following manufacturers instructions. Alterations in oligonucleotide concentrations can be employed to determine the optimum concentration of oligonucleotide according to the procedures described herein for any particular oligonucleotide of the invention.

Alternatively, the oligonucleotide of the invention may be delivered to a plant cell by electroporation of a protoplast derived from a plant part. The protoplasts may be formed by enzymatic treatment of a plant part, particularly a leaf, according to techniques such as those in Gallois et al., Methods in Molecular Biology 55: 89-107 by Humana Press. Such conditions for electroporation use

about 3 x 10<sup>5</sup> protoplasts in a total volume of about 0.3 ml with a concentration of oligonucleotide of between 0.6 to 4 microgram per ml.

#### **EXAMPLE 4**

#### **Plant Cells**

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The oligonucleotides of the invention can also be used to repair or direct a mutagenic event in plants and animal cells. Although little information is available on plant mutations amongst natural cultivars, the oligonucleotides of the invention can be used to produce "knock out" mutations by modification of specific amino acid codons to produce stop codons (e.g., a CAA codon specifying Gln can be modified at a specific site to TAA; a AAG codon specifying Lys can be modified to UAG at a specific site; and a CGA codon for Arg can be modified to a UGA codon at a specific site). Such base pair changes will terminate the reading frame and produce a defective truncated protein, shortened at the site of the stop codon

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Alternatively, frameshift additions or deletions can be directed into the genome at a specific sequence to interrupt the reading frame and produce a garbled downstream protein. Such stop or frameshift mutations can be introduced to determine the effect of knocking out the protein in either plant or animal cells.

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For introduction of a T-DNA, including the T-DNA in the plasmid of Figure 11, into a plant cell, *Agrobacterium tumefaciens* is used. These techniques are routine standard techniques known in the art. For example, one method follows. We transform *A. tumefaciens* is transformed by electroporation (using a BioRad Gene Pulser ). Competent *A. tumefaciens* is prepared using a method similar to that of preparing competent *E. coli* by suspending a freshly grown culture three times in ice-cold water and a final resuspension in 10% glycerol. Electroporation conditions are a 0.2 cm gap cuvette at a setting of 25  $\mu$ F, 200  $\Omega$  and 2.5 kV.

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A. tumefaciens containing a plasmid with a T-DNA is then used to introduce the T-DNA into a plant cell using routine standard techniques known in the art. For example, we transform Arabidopsis by vacuum infiltration or by dipping flowers in an Agrobacterium solution containing a surfactant, e.g. L-77. Seeds are then collected, grown and screened for presence of the T-DNA. Alternatively, Agrobacterium can be used to transform callus tissue and the callus tissue can then be used to regenerate transformed plants.

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All publications and patent applications cited in this specification are herein incorporated by reference as if each individual publication or patent application were specifically and individually indicated to be incorporated by reference. Although the foregoing invention has been described in some

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detail by way of illustration and example for purposes of clarity of understanding, it will be readily apparent to those of ordinary skill in the art in light of the teachings of this invention that certain changes and modifications may be made thereto without departing from the spirit or scope of the appended claims.

#### Notes on the tables presented below:

Each of the following tables presents, for the specified gene, a plurality of mutations that are known to confer a relevant phenotype and, for each mutation, the oligonucleotides that can be used to correct the respective mutation site-specifically in the genome according to the present invention.

The left-most column identifies each alteration or mutation and the phenotype that the alteration/mutation confers.

For most entries, the mutation/alteration is identified at both the nucleic acid and protein level. At the amino acid level, mutations are presented according to the following standard nomenclature. The centered number identifies the position of the mutated codon in the protein sequence; to the left of the number is the wild type residue and to the right of the number is the mutant codon. Terminator codons are shown as "TERM". At the nucleic acid level, the entire triplet of the wild type and mutated codons is shown.

The middle column presents, for each mutation, four oligonucleotides capable of repairing the mutation site-specifically in the genome or in cloned DNA including DNA in artificial chromosomes, episomes, plasmids, or other types of vectors. The oligonucleotides of the invention, however, may include any of the oligonucleotides sharing portions of the sequence of the 121 base sequence. Thus, oligonucleotides of the invention for each of the depicted targets may be 18, 19, 20 up to about 121 nucleotides in length. Sequence may be added non-symmetrically.

All oligonucleotides are presented, per convention, in the 5' to 3' orientation. The nucleotide that effects the change in the genome is underlined and presented in bold.

The first of the four oligonucleotides for each mutation is a 121 nt oligonucleotide centered about the repair/altering nucleotide. The second oligonucleotide, its reverse complement, targets the opposite strand of the DNA duplex for repair/alteration. The third oligonucleotide is the minimal 17 nt domain of the first oligonucleotide, also centered about the repair/alteration nucleotide. The fourth oligonucleotide is the reverse complement of the third, and thus represents the minimal 17 nt domain of the second.

The third column of each table presents the SEQ ID NO: of the respective repair oligonucleotide.

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## Example 5 Engineering herbicide resistant plants

Chemical weed control is an important tool of modern agriculture and many herbicides have been developed for this purpose. Their use has resulted in substantial increases in the yields of many crops, including, for example, maize, soybeans, and cotton. Thus while the use of fertilizers and new high-yielding crop varieties have contributed greatly to the "green revolution," chemical weed control has also been at the forefront of technological achievement.

Herbicides having broad-spectrum activity are particularly useful because they obviate the need for multiple herbicides targeting different classes of weeds. The problem with such herbicides is that they typically also affect crops which are exposed to the herbicide. One way to overcome this is to generate plants which are resistant to one or more broad-spectrum herbicides. Such herbicide-tolerant plants may reduce the need for tillage to control weeds, thereby effectively reducing soil erosion and can reduce the quantity and number of different herbicides applied in the field.

Common herbicides used, for example, include those that inhibit the enzyme 5-enolpyruvyl-3-phosphoshikimic acid synthase (EPSPS), for example N-phosphonomethyl-glycine (e.g. glyphosate), those that inhibit acetolactate synthase (ALS) activity, for example the sulfonylureas and related herbicides, and those that inhibit dihydropteroate synthase, for example methyl[(4-aminophenyl)sulfonyl]carbamate (e.g. Asulam). Herbicide-tolerant plants can be produced by several methods, including, for example, introducing into the genome of the plant the ability to degrade the herbicide, the capacity to produce a higher level of the targeted enzyme, and/or expressing an herbicide-tolerant allele of the enzyme.

The attached tables disclose exemplary oligonucleotides base sequences which can be used to generate site-specific mutations in plant genes that confer herbicide resistance.

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Table 10

Genome-Altering Oligos Conferring Glyphosate Resistance

Phenotype, Gene, Plant & Targeted	Altering Oligos	SEQID
Atteration		NO:
Glyphosate Resistance	AAGCGTCGGAGATTGTACTTCAACCCATTAGAGAAATCTCCGGTC	4341
EPSPS	TTATTAAGCTTCCTGCCTCCAAGTCTCTATCAAATCGGATCCTGC	
Arabidopsis thaliana	TTCTCGCTGCTCTGAGGTATATATCAC	
Gly97Ala	GTGATATATACCTCAGACAGAGCAGCAGAGAAGCAGGATCCGATT	4342
GGC-GCC	TGATAGAGACTTGGAGGCAGGAAGCTTAATAAGACCGGAGATTT	
	CTCTAATGGGTTGAAGTACAATCTCCGACGCTT	10.10
	GCTTCCTG <u>C</u> CTCCAAGT	4343
·	ACTTGGAGGCAAGC	4344
Glyphosate Resistance	AAGCTTCAGAGATTGTGCTTCAACCAATCAGAGAAATCTCGGGTC	4345
EPSPS	TCATTAAGCTACCCG <u>C</u> ATCCAAATCTCTCTCCAATCGGATCCTCC	
Brassica napus	TTCTTGCCGCTCTATCTGAGGTACATATACT	
Gly93Ala	AGTATATGTACCTCAGATAGAGCGGCAAGAAGGAGGATCCGATT	4346
GGA-GCA	GGAGAGAGATTTGGAT <u>G</u> CGGGTAGCTTAATGAGACCCGAGATTT	
	CTCTGATTGGTTGAAGCACAATCTCTGAAGCTT	
	GCTACCCG <u>C</u> ATCCAAAT	4347
	ATTTGGAT <u>G</u> CGGGTAGC	4348
Glyphosate Resistance	AGCCCAACGAGATTGTGCTGCAACCCATCAAAGATATATCAGGC	4349
EPSPS 1	ACTGTTAAATTGCCTGCTTCTAAATCCCTTTCCAATCGTATTCTCC	
Nicotiana tabacum	TTCTTGCTGCCCTTTCTAAGGGAAGGACTGT	
Gly95Ala	ACAGTCCTTCCCTTAGAAAGGGCAGCAAGAAGGAGAATACGATT	4350
GGT-GCT	GGAAAGGGATTTAGAA <u>G</u> CAGGCAATTTAACAGTGCCTGATATATC	
	TTTGATGGGTTGCAGCACAATCTCGTTGGGCT	
	ATTGCCTGCTTCTAAAT	4351
	ATTTAGAA <u>G</u> CAGGCAAT	4352
Glyphosate Resistance	ATTGTTTCCTTGGTACGAAATGTCCTCCTGTTCGAATTGTCAGCA	4353
EPSPS 2	AGGGAGGCCTTCCCG <u>C</u> AGGGAAGGTAAAGCTCTCTGGATCAATT	
Nicotiana tabacum	AGCAGCCAGTACTTGACTGCTCTGCTTATGGC	
Gly62Ala	GCCATAAGCAGAGCAGTCAAGTACTGGCTGCTAATTGATCCAGA	4354
GGA-GCA	GAGCTTTACCTTCCCTGCGGGAAGGCCTCCCTTGCTGACAATTC	
	GAACAGGAGGACATTTCGTACCAAGGAAACAAT	
	CCTTCCCG <u>C</u> AGGGAAGG	4355
	CCTTCCCT <u>G</u> CGGGAAGG	4356
Glyphosate Resistance	ATTGTTTCCTTGGCACTGACTGCCCACCTGTTCGTGTCAATGGAA	4357
EPSPS	TCGGAGGGCTACCTGCTGGCAAGGTCAAGCTGTCTGGCTCCATC	
Zea mays	AGCAGTCAGTACTTGAGTGCCTTGCTGATGGC	
Gly168Ala	GCCATCAGCAAGGCACTCAAGTACTGACTGCTGATGGAGCCAGA	4358
GGT-GCT	CAGCTTGACCTTGCCAGCAGGTAGCCCTCCGATTCCATTGACAC	
	GAACAGGTGGCAGTCAGTGCCAAGGAAACAAT	

Phenotype, Gene, Plant & Targeted		SEQ ID
Alteration	Altering Oligos	NO:
	GCTACCTG <u>C</u> TGGCAAGG	4359
	CCTTGCCA <u>G</u> CAGGTAGC	4360
Glyphosate Resistance	ACTGTTTCCTTGGCACTGAATGCCCACCTGTTCGTGTCAAGGGA	4361
EPSPS	ATTGGAGGACTTCCTGCTGGCAAGGTTAAGCTCTCTGGTTCCAT	1001
Oryza sativa	CAGCAGTCAGTACTTGAGTGCCTTGCTGATGGC	.
Gly115Ala	GCCATCAGCAAGGCACTCAAGTACTGACTGCTGATGGAACCAGA	4362
GGT-GCT	GAGCTTAACCTTGCCAGCAGGAAGTCCTCCAATTCCCTTGACAC	
	GAACAGGTGGCATTCAGTGCCAAGGAAACAGT	
	ACTTCCTG <u>C</u> TGGCAAGG	4363
	CCTTGCCA <u>G</u> CAGGAAGT	4364
Glyphosate Resistance		4365
EPSPS	CTGTTAAATTGCCTGCCTCTAAATCATTATCTAATAGAATTCTCCT	
Petunia x hybrida	TCTTGCTGCCTTATCTGAAGGAACAACTGT	ĺ
Gly93Ala GGC-GCC	ACAGTTGTTCCTTCAGATAAGGCAGCAAGAAGGAGAATTCTATTA	4366
GGC-GCC	GATAATGATTTAGAGGCAGGCAATTTAACAGTGCCTGAAATCTCT	
	TTAATGGGTTGCAACACTATCTCAGAAGGCT	
	ATTGCCTG <u>C</u> CTCTAAAT	4367
	ATTTAGAG <u>G</u> CAGGCAAT	4368
Glyphosate Resistance	AACCCCATGAGATTGTGCTAGNACCCATCAAAGATATATCTGGTA	4369
EPSPS	CTGTTAAATTACCCGCTTCGAAATCCCTTTCCAATCGTATTCTCCT	
Lycopersicon	TCTTGCTGCCCTTTCTGAGGGAAGGACTGT	
esculentum Gly97Ala	ACAGTCCTTCCCTCAGAAAGGGCAGCAAGAAGGAGAATACGATT	4370
GGT-GCT	GGAAAGGGATTTCGAAGCGGGTAATTTAACAGTACCAGATATATC	i
001-001	TTTGATGGGTNCTAGCACAATCTCATGGGGTT	
	ATTACCCGCTTCGAAAT	4371
	ATTTCGAAGCGGGTAAT	4372
Clyphocato Booistages		-1072
Glyphosate Resistance EPSPS	ATTGTTTCCTTGGCACTGACTGCCCACCTGTTCGKATCAACGGCA	4373
Lolium rigidum	TTGGAGGGCTACCTGCTGGCAAGGTTAAGCTGTCTGGTTCCATC	Ì
Gly107Ala	AGCAGCAACTAACTAACTAACTAACTAACTAACTAACTAA	
GGT-GCT	GCCATCAGCAAGGAACTCAAGTATTGGCTGCTGATGGAACCAGA	4374
	CAGCTTAACCTTGCCAGCAGGTAGCCCTCCAATGCCGTTGATCG AACAGGTGGCCAGTCAGTGCCAAGGAAACAAT	1
	GCTACCTGCTGCCAAGGAAACAAT	4055
}		4375
	CCTTGCCAGCAGGTAGC	4376

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Table 11

Genome-Altering Oligos Conferring Imidazolinone and Sulfonylurea Herbicide Resistance

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Sulfonylurea Resistance ALS	AGCGGATTAGCCGATGCGTTGTTAGATAGTGTTCCTCTTGTAGCA ATCACAGGACAAGTCTCTCGTCGTATGATTGGTACAGATGCGTTT CAAGAGACTCCGATTGTTGAGGTAACGCGTT	4377
Arabidopsis thaliana Pro197Ser CCT-TCT	AACGCGTTACCTCAACAATCGGAGTCTCTTGAAACGCATCTGTAC CAATCATACGACGAGAGACTTGTCCTGTGATTGCTACAAGAGGAA CACTATCTAACAACGCATCGGCTAATCCGCT	4378
	GACAAGTCTCTCGTCGT .	4379
	ACGACGAG <u>A</u> GACTTGTC	4380
Sulfonylurea Resistance ALS	AGCGGATTAGCCGATGCGTTGTTAGATAGTGTTCCTCTTGTAGCA ATCACAGGACAAGTCCAGCGTCGTATGATTGGTACAGATGCGTTT CAAGAGACTCCGATTGTTGAGGTAACGCGTT	4381
Arabidopsis thaliana Pro197Gln CCT-CAG	AACGCGTTACCTCAACAATCGGAGTCTCTTGAAACGCATCTGTAC CAATCATACGACGCTGGACTTGTCCTGTGATTGCTACAAGAGGAA CACTATCTAACAACGCATCGGCTAATCCGCT	4382
	ACAAGTCC <u>AG</u> CGTCGTC	4383
	TACGACG <u>CT</u> GGACTTGT	4384
Sulfonylurea Resistance ALS	AGCGGATTAGCCGATGCGTTGTTAGATAGTGTTCCTCTTGTAGCA ATCACAGGACAAGTCC <u>AA</u> CGTCGTATGATTGGTACAGATGCGTTT CAAGAGACTCCGATTGTTGAGGTAACGCGTT	4385
Arabidopsis thaliana Pro197Gln CCT-CAA	AACGCGTTACCTCAACAATCGGAGTCTCTTGAAACGCATCTGTAC CAATCATACGACGTTGGACTTGTCCTGTGATTGCTACAAGAGGAA CACTATCTAACAACGCATCGGCTAATCCGCT	4386
	ACAAGTCC <u>AA</u> CGTCGTA	4387
	TACGACGTTGGACTTGT	4388
Imidazolinone Resistance ALS	GACCITACCTGTTGGATGTGATTTGTCCGCACCAAGAACATGTGT TGCCGATGATCCCGAACGGTGGCACTTTCAACGATGTCATAACGG AAGGAGATGGCCGGATTAAATACTGAGAGAT	4389
Arabidopsis thaliana Ser653Asn AGT-AAC	ATCTCTCAGTATTTAATCCGGCCATCTCCTTCCGTTATGACATCGT TGAAAGTGCCACCGTTCGGGATCATCGGCAACACATGTTCTTGGT GCGGACAAATCACATCCAACAGGTAAGGTC	4390
<del></del>	GATCCCGA <u>AC</u> GGTGGCA	4391
	TGCCACC <u>GT</u> TCGGGATC	4392

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ III
Imidazolinone Resistance ALS Arabidopsis thaliana	GACCTTACCTGTTGGATGTGATTTGTCCGCACCAAGAACATGTGT TGCCGATGATCCCGAATGGTGGCACTTTCAACGATGTCATAACGG AAGGAGATGGCCGGATTAAATACTGAGAGAT	
Ser653Asn AGT-AAT	ATCTCTCAGTATTTAATCCGGCCATCTCCTTCCGTTATGACATCGT TGAAAGTGCCACCATCGGGATCATCGGCAACACATGTTCTTGGT GCGGACAAATCACATCCAACAGGTAAGGTC	4394
	GATCCCGA <u>AT</u> GGTGGCA	4395
Culfondure	TGCCACC <u>AT</u> TCGGGATC	4396
Sulfonylurea Resistance ALS Oryza sativa	TCCGCGCTCGCCGACGCGCTGCTCGACTCCGATGGTCG CCATCACGGGCCAGGTCTCCCGCCGCATGATCGGCACCGACGC CTTCCAGGAGACGCCCATAGTCGAGGTCACCCGCT	4397
Pro171Ser CCC-TCC	AGCGGGTGACCTCGACTATGGGCGTCTCCTGGAAGGCGTCGGTG CCGATCATGCGGCGGGAGACCTGGCCCGTGATGGCGACCATCG GGACGAGTCGAGCAGCGCGTCGGCGAGCGCGGA GCCAGGTCTCCCGCCGC	4398 .
		4399
Culfonda	GCGGCGG <u>A</u> GACCTGGC	4400
Sulfonylurea Resistance ALS	CCGCGCTCGCCGACGCGCTGCTCGACTCCGTCCCGATGGTCGC CATCACGGGCCAGGTCCAACGCCGCATGATCGGCACCGACGCC TTCCAGGAGACGCCCATAGTCGAGGTCACCCGCTC	4401
<i>Oryza sativa</i> Pro171GIn CCC-CAA	GAGCGGGTGACCTCGACTATGGGCGTCTCTCTGGAAGGCGTCGGT GCCGATCATGCGGCGTTGGACCTGGCCCGTGATGGCGACCATCG GGACGGAGTCGAGCAGCGCGCGGGGGCGCGG	4402
	CCAGGTCC <u>AA</u> CGCCGCA	4403
	TGCGGCG <u>TT</u> GGACCTGG	4404
Sulfonylurea Resistance ALS	CCGCGCTCGCCGACGCGCTGCTCGACTCCGATGGTCGC CATCACGGGCCAGGTCCAGCGCCCATGATCGGCACCGACGCC TTCCAGGAGACGCCCATAGTCGAGGTCACCCGCTC	4405
Oryza sativa Pro171GIn CCC-CAG	GAGCGGGTGACCTCGACTATGGGCGTCTCCTGGAAGGCGTCGGT GCCGATCATGCGGCGCTGGACCTGGCCCGTGATGGCGACCATC GGGACGGAGTCGAGCAGCGCGCGCGGGGGCGCGG	4406
	CCAGGTCC <u>AG</u> CGCCGCA	4407
	TGCGGCG <u>CT</u> GGACCTGG	4408
nidazolinone lesistance LS	GGCCATACTTGTTGGATATCATCGTCCCGCACCAGGAGCATGTGC TGCCTATGATCCCAAATGGGGGGCGCATTCAAGGACATGATCCTGG ATGGTGATGGCAGGACTGTGTATTAATCTAT	4409
<i>ryza sativa</i> e627Asn TT-AAT	ATAGATTAATACACAGTCCTGCCATCACCATCCAGGATCATGTCCT TGAATGCGCCCCCATTTGGGATCATAGGCAGCACATGCTCCTGGT GCGGGACGATGATATCCAACAAGTATGGCC	4410
	GATCCCAAATGGGGGCG	4411

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID
	CGCCCCA <u>T</u> TTGGGATC	4412
Sulfonylurea Resistance	TCCGCGCTCGCCGACGCGCTGCTCGATTCCGTCCCCATGGTCGC CATCACGGGACAGGTGTCGCGACGCATGATTGGCACCGACGCCT TCCAGGAGACGCCCATCGTCGAGGTCACCCGCT	4413
ALS Zea mays Pro165Ser	AGCGGGTGACCTCGACGATGGCGTCTCCTGGAAGGCGTCGGT GCCAATCATGCGTCGCGACACCTGTCCCGTGATGGCGACCATGG GGACGGAATCGAGCAGCGCGTCGGCGAGCGCGGA	4414
CCG-TCG	GACAGGTGTCGCGACGC	4415
•	GCGTCGCGACACCTGTC	4416
Sulfonylurea Resistance ALS	CCGCGCTCGCCGACGCGCTGCTCGATTCCGTCCCCATGGTCGCC ATCACGGGACAGGTGCAGCGACGCATGATTGGCACCGACGCCTT CCAGGAGACGCCCATCGTCGAGGTCACCCGCTC	4417
Zea mays Pro165Gin CCG-CAG	GAGCGGTGACCTCGACGATGGGCGTCTCCTGGAAGGCGTCGG TGCCAATCATGCGTCGCTGCACCTGTCCCGTGATGGCGACCATG GGGACGGAATCGAGCAGCGCGTCGGCGAGCGCGG	4418
CCG-CAG	ACAGGTGCAGCGCA	4419
	TGCGTCGC <u>T</u> GCACCTGT	4420
Imidazolinone Resistance ALS	GGCCGTACCTCTTGGATATAATCGTCCCACACCAGGAGCATGTGT TGCCTATGATCCCTAATGGTGGGGCTTTCAAGGATATGATCCTGG ATGGTGATGGCAGGACTGTGTACTGATCTAA	4421
Zea mays Ser621Asn AGT-AAT	TTAGATCAGTACACAGTCCTGCCATCACCATCCAGGATCATATCCT TGAAAGCCCCACCATTAGGGATCATAGGCAACACATGCTCCTGGT GTGGGACGATTATATCCAAGAGGTACGGCC	4422
AGI-AAI	GATCCTAATGGTGGGG	4423
	CCCCACC <u>AT</u> TAGGGATC	4424
Imidazolinone Resistance ALS	GGCCGTACCTCTTGGATATAATCGTCCCACACCAGGAGCATGTGT TGCCTATGATCCCTAACGGTGGGGCTTTCAAGGATATGATCCTGG ATGGTGATGGCAGGACTGTGTACTGATCTAA	4425
Zea mays Ser621Asn AGT-AAC	TTAGATCAGTACACAGTCCTGCCATCACCATCCAGGATCATATCCT TGAAAGCCCCACCGTTAGGGATCATAGGCAACACATGCTCCTGGT GTGGGACGATTATATCCAAGAGGTACGGCC	4426
AGI-AAC	GATCCTA <u>AC</u> GGTGGGG	4427
	CCCCACC <u>GT</u> TAGGGATC	4428
Sulfonylurea Resistance	TCCGCGCTCGCCGACGCCCTCCTCGACTCCCATCGCTGGCCCATCACGGGGCAGGTCTCGCGCCGCATGATCGGCACGGACGCCCTTCCAGGAGACGCCCATCGTCGAGGTCACCCGCT	4429
ALS Lolium multiflorum Pro167Ser CCG-TCG	AGCGGTGACCTCGACGATGGCGTCTCCTGGAAGGCGTCCGT GCCGATCATGCGGCGCGAGACCTGCCCCGTGATGGCCACCATG GGGATGGAGTCGAGGAGGGCGTCGGCGAGCGCGGA	4430

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	GGCAGGTC <u>T</u> CGCGCCGC	4431
	GCGGCGC <u>A</u> GACCTGCC	4432
Sulfonylurea Resistance ALS	CCGCGCTCGCCGACGCCCTCCTCGACTCCATCCCCATGGTGGCC ATCACGGGGCAGGTCCAGCCCCCATGATCGGCACGCCCT TCCAGGAGACGCCCATCGTCGAGGTCACCCGCTC	4433
Lolium multiflorum Pro167Gln CCG-CAG	GAGCGGTGACCTCGACGATGGCCGTCTCCTGGAAGGCGTCCG TGCCGATCATGCGGCGCTGGACCTGCCCCGTGATGGCCACCATG GGGATGGAGTCGAGGAGGGCGTCGCCGAGCGCGG	4434
	GCAGGTCC <u>A</u> GCGCCGCA	4435
	TGCGGCGCTGGACCTGC	4436
Imidazolinone Resistance ALS	CTGGGCCATACTTGTTGGATATCATCGTCCCTCACCAGGAGCATG TGCTGCCTATGATCCCTAACGGTGGTGCTTTCAAGGACATTATCA TGGAAGGTGATGGCAGGATTTCGTATTAAAC	4437
Lolium multiflorum Ser623Asn AGC-AAC	GTTTAATACGAAATCCTGCCATCACCTTCCATGATAATGTCCTTGA AAGCACCACCGTTAGGGATCATAGGCAGCACATGCTCCTGGTGA GGGACGATGATATCCAACAAGTATGGCCCAG	4438
	GATCCCTA <u>A</u> CGGTGGTG	4439
	CACCACCG <u>T</u> TAGGGATC	4440
Sulfonylurea Resistance ALS	TCCGCGCTCGCCGACGCTCTCCTCGACTCCATCCCCATGGTCGC CATCACGGGCCAGGTCTCACGCCGCATGATCGGCACGGACGCGT TCCAGGAGACGCCCATAGTGGAGGTCACGCGCT	4441
Hordeum vulgare Pro68Ser CCA-TCA	AGCGCGTGACCTCCACTATGGGCGTCCTGGAACGCGTCCGTGCCGATCATGCGCGCGTGATGGCGACCATGGCGATGGAGTCGAGGAGGAGCGTCGGCGAGCGCGA	4442
	GCCAGGTC <u>T</u> CACGCCGC	4443
	GCGCGTG <u>A</u> GACCTGGC	4444
Sulfonylurea Resistance ALS	CCGCGCTCGCCGACGCTCTCCTCGACTCCATCCCCATGGTCGCC ATCACGGGCCAGGTCCAACGCCGCATGATCGGCACGGACGCGTT CCAGGAGACGCCCATAGTGGAGGTCACGCGCTC	4445
Hordeum vulgare Pro68Gln CCA-CAA	GAGCGCGTGACCTCCACTATGGGCGTCTCTGGAACGCGTCCGT GCCGATCATGCGGCGTTGGACCTGGCCCGTGATGGCGACCATGG GGATGGAGTCGAGGAGAGCGTCGGCGAGCGCGG	4446
	CCAGGTCC <u>A</u> ACGCCGCA	4447
	TGCGGCGT <u>T</u> GGACCTGG	4448

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Imidazolinone Resistance ALS	CCCAGGGCCGTACCTGCTGGATATCATTGTCCCGCATCAGGAGC ACGTGCTGCCTATGATCCCAAACGGTGGTGCTTTCAAGGACATGA TCATGGAGGGTGATGGCAGGACCTCGTACTGA	4449
Hordeum vulgare Ser524Asn AGC-AAC	TCAGTACGAGGTCCTGCCATCACCCTCCATGATCATGTCCTTGAA AGCACCACCGTTTGGGATCATAGGCAGCACGTGCTCCTGATGCG GGACAATGATATCCAGCAGGTACGGCCCTGGG	4450
	GATCCCAA <u>A</u> CGGTGGTG	4451
	CACCACCG <u>T</u> TTGGGATC	4452
Sulfonylurea Resistance ALS	AGTGGTCTCGCTGATGCAATGCTCGATAGTATCCCTCTCGTGGCG ATCACTGGTCAAGTCTCTCGTCGGATGATCGGTACCGATGCTTTC CAGGAAACTCCAATTGTTGAGGTAACAAGGT	4453
Gossypium hirsutum Pro186Ser CCT-TCT	ACCTTGTTACCTCAACAATTGGAGTTTCCTGGAAAGCATCGGTAC CGATCATCCGACGAGAGACCTTGACCAGTGATCGCCACGAGAGGGGATACTATCGAGCATTGCATCAGCGAGACCACT	4454
	GTCAAGTC <u>T</u> CTCGTCGG	4455
	CCGACGAG <u>A</u> GACTTGAC	4456
Sulfonylurea Resistance ALS	GTGGTCTCGCTGATGCAATGCTCGATAGTATCCCTCTCGTGGCGA TCACTGGTCAAGTCCAACGTCGGATGATCGGTACCGATGCTTTCC AGGAAACTCCAATTGTTGAGGTAACAAGGTC	4457
Gossypium hirsutum Pro186Gln CCT-CAA	GACCTTGTTACCTCAACAATTGGAGTTTCCTGGAAAGCATCGGTA CCGATCATCCGACG <u>TT</u> GGACTTGACCAGTGATCGCCACGAGAGG GATACTATCGAGCATTGCATCAGCGAGACCAC	4458
	TCAAGTCC <u>AA</u> CGTCGGA	4459
	TCCGACG <u>TT</u> GGACTTGA	4460
Sulfonylurea Resistance ALS	GTGGTCTCGCTGATGCAATGCTCGATAGTATCCCTCTCGTGGCGA TCACTGGTCAAGTCCAGCGTCGGATGATCGGTACCGATGCTTTCC AGGAAACTCCAATTGTTGAGGTAACAAGGTC	4461
Gossypium hirsutum Pro186Gin CCT-CAG	GACCTTGTTACCTCAACAATTGGAGTTTCCTGGAAAGCATCGGTA CCGATCATCCGACGCTGGACTTGACCAGTGATCGCCACGAGAGG GATACTATCGAGCATTGCATCAGCGAGACCAC	4462
	TCAAGTCC <u>AG</u> CGTCGGA	4463
	TCCGACG <u>CT</u> GGACTTGA	4464
Imidazolinone Resistance ALS	GACCTTACTTGTTGGATGTGATTGTCCCACATCAAGAACATGTCCT GCCTATGATCCCCAATGGAGGCGCTTTCAAAGATGTGATCACAGA GGGTGATGGAAGAACACAATATTGACCTCA	4465
Gossypium hirsutum Ser642Asn AGT-AAT	TGAGGTCAATATTGTGTTCTTCCATCACCCTCTGTGATCACATCTT TGAAAGCGCCTCCATTGGGGATCATAGGCAGGACATGTTCTTGAT GTGGGACAATCACATCCAACAAGTAAGGTC	4466
	GATCCCCAATGGAGGCG	4467

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID
	CGCCTCCATTGGGGATC	4468
Sulfonylurea Resistance ALS Amaranthus	TCTGGTCTTGCTGATGCACTTCTTGACTCAGTCCCTCTTGTCGCC ATTACTGGGCAAGTTTCCCGGCGTATGATTGGTACTGATGCTTTTC AAGAGACTCCAATTGTTGAGGTAACTCGAT	4469
retroflexus Pro192Ser CCC-TCC	ATCGAGTTACCTCAACAATTGGAGTCTCTTGAAAAGCATCAGTACC AATCATACGCCGGGAAACTTGCCCAGTAATGGCGACAAGAGGGA CTGAGTCAAGAAGTGCATCAGCAAGACCAGA GGCAAGTTTCCCGGCGT	4470
		4471
	ACGCCGGG <u>A</u> AACTTGCC	4472
Sulfonylurea Resistance ALS	CTGGTCTTGCTGATGCACTTCTTGACTCAGTCCCTCTTGTCGCCA TTACTGGGCAAGTTCAACGGCGTATGATTGGTACTGATGCTTTTC AAGAGACTCCAATTGTTGAGGTAACTCGATC	4473
Amaranthus retroflexus Pro192Gin CCC-CAA	GATCGAGTTACCTCAACAATTGGAGTCTCTTGAAAAGCATCAGTAC CAATCATACGCCG <u>TT</u> GAACTTGCCCAGTAATGGCGACAAGAGGGA CTGAGTCAAGAAGTGCATCAGCAAGACCAG	4474
000-044	GCAAGTTC <u>AA</u> CGGCGTA	4475
	TACGCCGTTGAACTTGC	4476
Sulfonylurea Resistance ALS	CTGGTCTTGCTGATGCACTTCTTGACTCAGTCCCTCTTGTCGCCA TTACTGGGCAAGTTCAGCGGCGTATGATTGGTACTGATGCTTTTC AAGAGACTCCAATTGTTGAGGTAACTCGATC	4477
Amaranthus retroflexus Pro192Gin CCC-CAG	GATCGAGTTACCTCAACAATTGGAGTCTCTTGAAAAGCATCAGTAC CAATCATACGCCG <u>CT</u> GAACTTGCCCAGTAATGGCGACAAGAGGG ACTGAGTCAAGAAGTGCATCAGCAAGACCAG	4478
CCC-CAG	GCAAGTTC <u>AG</u> CGGCGTA	4479
	TACGCCG <u>CT</u> GAACTTGC	4480
Imidazolinone Resistance ALS Amaranthus	GACCGTATCTGCTGGATGTAATCGTACCACATCAGGAGCATGTGC TGCCTATGATCCCTAACGGTGCCGCCTTCAAGGACACCATAACAG AGGGTGATGGAAGAAGGGCTTATTAGTTGGT	4481
retroflexus Ser652Asn AGC-AAC	ACCAACTAATAAGCCCTTCTTCCATCACCCTCTGTTATGGTGTCCT TGAAGGCGGCACCGTTAGGGATCATAGGCAGCACATGCTCCTGA TGTGGTACGATTACATCCAGCAGATACGGTC	4482
	GATCCCTA <u>A</u> CGGTGCCG	4483
	CGGCACCGTTAGGGATC	4484

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Sulfonylurea Resistance ALS 1	AGCGGCCTCGCTGACGCGCTACTGGATAGCGTCCCCATTGTTGC TATAACAGGTCAAGTGTCACGTAGGATGATAGGTACTGATGCTTTT CAGGAAACTCCTATTGTTGAGGTAACTAGAT	4485
Nicotiana tabacum Pro194Ser CCA-TCA	ATCTAGTTACCTCAACAATAGGAGTTTCCTGAAAAGCATCAGTACC TATCATCCTACGTGACACTTGACCTGTTATAGCAACAATGGGGAC GCTATCCAGTAGCGCGTCAGCGAGGCCGCT	4486
	GTCAAGTG <u>T</u> CACGTAGG	4487
·	CCTACGTG <u>A</u> CACTTGAC	4488
Sulfonylurea Resistance ALS 1	GCGGCCTCGCTGACGCGCTACTGGATAGCGTCCCCATTGTTGCT ATAACAGGTCAAGTGCAACGTAGGATGATAGGTACTGATGCTTTT CAGGAAACTCCTATTGTTGAGGTAACTAGATC	4489
Nicotiana tabacum Pro194Gln CCA-CAA	GATCTAGTTACCTCAACAATAGGAGTTTCCTGAAAAGCATCAGTAC CTATCATCCTACGTTGCACTTGACCTGTTATAGCAACAATGGGGA CGCTATCCAGTAGCGCGTCAGCGAGGCCGC	4490
	TCAAGTGC <u>A</u> ACGTAGGA	4491
	TCCTACGTTGCACTTGA	4492
Imidazolinone Resistance ALS 1	GGCCATACTTGTTGGATGTGATTGTACCTCATCAGGAACATGTTTT ACCTATGATTCCCAATGGCGGAGCTTTCAAAGATGTGATCACAGA GGGTGACGGGAGAAGTTCCTATTGAGTTTG	4493
Nicotiana tabacum Ser650Asn AGT-AAT	CAAACTCAATAGGAACTTCTCCCGTCACCCTCTGTGATCACATCTT TGAAAGCTCCGCCATTGGGAATCATAGGTAAAACATGTTCCTGAT GAGGTACAATCACATCCAACAAGTATGGCC	4494
	GATTCCCA <u>A</u> TGGCGGAG	4495
	CTCCGCCA <u>T</u> TGGGAATC	4496
Sulfonylurea Resistance ALS 2	AGTGGCCTCGCGGACGCCCTACTGGATAGCGTCCCCATTGTTGC TATAACCGGTCAAGTGTCACGTAGGATGATCGGTACTGATGCTTTT CAGGAAACTCCGATTGTTGAGGTAACTAGAT	4497
Nicotiana tabacum Pro191Ser CCA-TCA	ATCTAGTTACCTCAACAATCGGAGTTTCCTGAAAAGCATCAGTACC GATCATCCTACGTGACACTTGACCGGTTATAGCAACAATGGGGAC GCTATCCAGTAGGGCGTCCGCGAGGCCACT	4498
	GTCAAGTG <u>T</u> CACGTAGG	4499
	CCTACGTG <u>A</u> CACTTGAC	4500
Sulfonylurea Resistance ALS 2	GTGGCCTCGCGGACGCCCTACTGGATAGCGTCCCCATTGTTGCT ATAACCGGTCAAGTGCAACGTAGGATGATCGGTACTGATGCTTTT CAGGAAACTCCGATTGTTGAGGTAACTAGATC	4501
Nicotiana tabacum Pro191Gin CCA-CAA	GATCTAGTTACCTCAACAATCGGAGTTTCCTGAAAAGCATCAGTAC CGATCATCCTACGTTGCACTTGACCGGTTATAGCAACAATGGGGA CGCTATCCAGTAGGGCGTCCGCGAGGCCAC	4502
	TCAAGTGC <u>A</u> ACGTAGGA	4503

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID
	TCCTACGT <u>T</u> GCACTTGA	4504
Imidazolinone Resistance	GGCCATACTTGTTGGATGTGATTGTACCTCATCAGGAACATGTTCT ACCTATGATTCCCAATGGCGGGGCTTTCAAAGATGTGATCACAGA	1
ALS 2	GGGTGACGGGAGAAGTTCCTATTGACTTTG	i .
Nicotiana tabacum Ser647Asn AGT-AAT	CAAAGTCAATAGGAACTTCTCCCGTCACCCTCTGTGATCACATCTT TGAAAGCCCCGCCATGGGAATCATAGGTAGAACATGTTCCTGAT GAGGTACAATCACATCCAACAAGTATGGCC	4506
	GATTCCCA <u>A</u> TGGCGGGG	4507
	CCCCGCCATTGGGAATC	4508
Sulfonylurea Resistance ALS	AGTGGTCTTGCTGATGCTTTATTAGACAGTGTTCCAATGGTTGCTA TTACTGGTCAAGTTTCCAGGAGAATGATTGGAACAGATGCGTTTCA AGAAACCCCTATTGTTGAGGTAACACGTT	4509
Xanthium spp. Pro175Ser CCC-TCC	AACGTGTTACCTCAACAATAGGGGTTTCTTGAAACGCATCTGTTCC AATCATTCTCCTGGAACTTGACCAGTAATAGCAACCATTGGAACA CTGTCTAATAAAGCATCAGCAAGACCACT	4510
	GTCAAGTTTCCAGGAGA	4511
	TCTCCTGGAAACTTGAC	4512
Sulfonylurea Resistance ALS	GTGGTCTTGCTGATGCTTTATTAGACAGTGTTCCAATGGTTGCTAT TACTGGTCAAGTTCAAAGGAGGAATGATTGGAACAGATGCGTTTCA AGAAACCCCTATTGTTGAGGTAACACGTTC	4513
Xanthium spp. Pro175Gin CCC-CAA	GAACGTGTTACCTCAACAATAGGGGTTTCTTGAAACGCATCTGTTC CAATCATTCTCCT <u>TT</u> GAACTTGACCAGTAATAGCAACCATTGGAAC ACTGTCTAATAAAGCATCAGCAAGACCAC	4514
	TCAAGTTC <u>AA</u> AGGAGAA	4515
	TTCTCCTTTGAACTTGA	4516
Sulfonylurea Resistance ALS	GTGGTCTTGCTGATGCTTTATTAGACAGTGTTCCAATGGTTGCTAT TACTGGTCAAGTTCAGAGAGAGAATGATTGGAACAGATGCGTTTCA AGAAACCCCTATTGTTGAGGTAACACGTTC	4517
Xanthium spp. Pro175GIn CCC-CAG	GAACGTGTTACCTCAACAATAGGGGTTTCTTGAAACGCATCTGTTC CAATCATTCTCCT <u>CT</u> GAACTTGACCAGTAATAGCAACCATTGGAAC ACTGTCTAATAAAGCATCAGCAAGACCAC	4518
	TCAAGTTC <u>AG</u> AGGAGAA	4519
	TTCTCCT <u>CT</u> GAACTTGA	4520
Imidazolinone Resistance ALS	GGGCCTTACTTGTTGGATGTGATCGTGCCCCATCAAGAACATGTG TTGCCCATGATCCCGAATGGTGGAGGTTTCATGGATGTGATCACC GAAGGCGACGCAGAATGAAATATTGAGCTT	4521
Alabatash	AAGCTCAATATTTCATTCTGCCGTCGCCTTCGGTGATCACATCCAT GAAACCTCCACCATTCGGGATCATGGGCAACACATGTTCTTGATG GGGCACGATCACATCCAACAAGTAAGGCCC	4522

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID
	TGATCCCG <u>AA</u> TGGTGGA	4523
	TCCACCA <u>TT</u> CGGGATCA	4524
Sulfonylurea Resistance	TCCGGGTTTGCTGATGCTTTGCTCGATTCCGTTCCACTGGTGGCG ATCACGGGGCAGGTGTCGCGGCGAATGATTGGGACGGATGCTTT	4525
ALS Bassia scoparia Pro189Ser	TCAGGAGACTCCTATTGTTGAGGTAACACGGT  ACCGTGTTACCTCAACAATAGGAGTCTCCTGAAAAGCATCCGTCC CAATCATTCGCCGCGACACCTGCCCCGTGATCGCCACCAGTGGA ACGGAATCGAGCAAAGCATCAGCAAACCCGGA	4526
CCG-TCG	GGCAGGTGTCGCGGCGA	4527
•	TCGCCGCGACACCTGCC	4528
Sulfonylurea Resistance	CCGGGTTTGCTGATGCTTTGCTCGATTCCGTTCCACTGGTGGCGA TCACGGGGCAGGTGCAGCGGCGAATGATTGGGACGGATGCTTTT CAGGAGACTCCTATTGTTGAGGTAACACGGTC	4529
ALS Bassia scoparia Pro189Gin	GACCGTGTTACCTCAACAATAGGAGTCTCCTGAAAAGCATCCGTC CCAATCATTCGCCGCTGCACCTGCCCCGTGATCGCCACCAGTGG AACGGAATCGAGCAAAGCATCAGCAAACCCGG	4530
CCG-CAG	GCAGGTGCAGCAAAGCATCAGCAAAGCAAAGCATCAGCAAAGCAAAGCATCAGCAAAGCAAAGCATCAGCAAAGCAAAGCATCAGCAAAGCAAAGCATCAGCAAAAGCAAAGCATCAGCAAAAGCAAAGCATCAGCAAAAGCAAAGCAAAGCAAAGCAAAGCAAAAGCAAAGCAAAGCAAAGCAAAGCAAAAAGCAAAAGCAAAAGCAAAAGCAAAAGCAAAAGCAAAAGCAAAAAGCAAAAAGCAAAAGCAAAAAGCAAAAAGCAAAAAGCAAAAAGCAAAAAGCAAAAAA	4531
	TTCGCCGCTGCACCTGC	4532
Imidazolinone Resistance	GACCTTACCTGCTTGATGTGATTGTACCTCATCAGGAGCATGTGC TGCCTATGATTCCTAATGGTGCAGCCTTCAAGGATATCATTAACGA AGGTGATGGAAGAACAAGTTATTGATGTTC	4533
ALS Bassia scoparia Ser649Asn AGT-AAT	GAACATCAATAACTTGTTCTTCCATCACCTTCGTTAATGATATCCTT GAAGGCTGCACCATTAGGAATCATAGGCAGCACATGCTCCTGATG AGGTACAATCACATCAAGCAGGTAAGGTC	4534
AGI-AAI	GATTCCTAATGGTGCAG	453
	CTGCACCATTAGGAATC	453
Sulfonylurea Resistance ALS 1	AGCGGGTTAGCAGACGCGATGCTTGACAGTGTTCCTCTTGTCGC CATTACAGGACAGG	453
Brassica napus Pro182Ser CCT-TCT	ACCTCGTTACCTCAACGATTGGTGTCTCTTGGAAGGCGTCAGTAC CGATCATCCGGCGAGAGACCTGTCCTGT	453
001-101	GACAGGTC <u>T</u> CTCGCCGG	453
	CCGGCGAGAGACCTGTC	454

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	Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID
	Sulfonylurea Resistance ALS 1 Brassica napus	GCGGGTTAGCAGACGCGATGCTTGACAGTGTTCCTCTTGTCGCC ATTACAGGACAGG	4541
5	Pro182GIn CCT-CAA	GACCTCGTTACCTCAACGATTGGTGTCTCTTGGAAGGCGTCAGTA CCGATCATCCGGCGTTGGACCTGTCCTGT	4542
		ACAGGTCC <u>AA</u> CGCCGGA TCCGGCG <u>TT</u> GGACCTGT	4543
	Culfondino		4544
10	Sulfonylurea Resistance ALS 1	GCGGGTTAGCAGACGCGATGCTTGACAGTGTTCCTCTTGTCGCC ATTACAGGACAGG	4545
10	Brassica napus Pro182GIn CCT-CAG	GACCTCGTTACCTCAACGATTGGTGTCTCTTGGAAGGCGTCAGTA CCGATCATCCGGCGCTGGACCTGTCCTGT	4546
		ACAGGTCC <u>AG</u> CGCCGGA	4547
		TCCGGCG <u>CT</u> GGACCTGT	4548
15	Imidazolinone Resistance ALS 1	GACCATACCTGTTGGATGTGATATGTCCGCACCAAGAACATGTGT TACCGATGATCCCAAATGGTGGCACTTTCAAAGATGTAATAACAGA AGGGGATGGTCGCACTAAGTACTGAGAGAT	4549
	Brassica napus Ser638Asn AGT-AAT	ATCTCTCAGTACTTAGTGCGACCATCCCCTTCTGTTATTACATCTT TGAAAGTGCCACCATITGGGATCATCGGTAACACATGTTCTTGGT GCGGACATATCACATCCAACAGGTATGGTC	4550
		GATCCCAA <u>A</u> TGGTGGCA	4551
		TGCCACCA <u>T</u> TTGGGATC	4552
20	Sulfonylurea Resistance ALS 2	CAGCGGGTTAGCAGACGCGATGCTTGACAGTGTTCCTCTTGTCG CCATTACAGGACAGG	4553
	Brassica napus Pro126Ser CCC-TCC	CCTCGTTACCTCAACGATTGGTGTCTCTTGGAAGGCGTCAGTACC GATCATCCGGCGAGGAACCTGTCCTGT	4554
		GGACAGGT <u>T</u> CCTCGCCG	4555
		CGGCGAGG <u>A</u> ACCTGTCC	4556
25	Sulfonylurea Resistance ALS 2	AGCGGGTTAGCAGACGCGATGCTTGACAGTGTTCCTCTTGTCGC CATTACAGGACAGG	4557
0	CCC-CAG	ACCTCGTTACCTCAACGATTGGTGTCTCTTGGAAGGCGTCAGTAC CGATCATCCGGCGAGTGACCTGTCCTGT	4558
•		GACAGGTC <u>A</u> CTCGCCGG	4559

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Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID
	CCGGCGAG <u>T</u> GACCTGTC	4560
midazolinone Resistance ALS 2	GACCATACCTGTTGGATGTGATATGTCCGCACCAAGAACATGTGT TACCGATGATCCCAAATGGTGGCACTTTCAAAGATGTAATAACAGA AGGGGATGGTCGCACTAAGTACTGAGAGAT	4561
ALG 2 B <i>rassica napus</i> Ser582Asn AGT-AAT	ATCTCTCAGTACTTAGTGCGACCATCCCCTTCTGTTATTACATCTT TGAAAGTGCCACCATTTGGGATCATCGGTAACACATGTTCTTGGT GCGGACATATCACATCCAACAGGTATGGTC	4562
NG1-7/1	GATCCAAATGGTGGCA	4563
	TGCCACCATTTGGGATC	4564
Sulfonylurea Resistance ALS 3	AGCGGGTTAGCCGACGCGATGCTTGACAGTGTTCCTCTCGCCCATCACAGGACAGGTCTCTCGCCGGATGATCGGTACTGACGCGTTCCCAAGAGACACCCCAATCGTTGAGGTAACGAGGT	4565
Brassica napus Pro179Ser CCT-TCT	ACCTCGTTACCTCAACGATTGGCGTCTCTTGGAACGCGTCAGTAC CGATCATCCGGCGAGAGACCTGTCCTGT	4566
Colston	GACAGGTC <u>T</u> CTCGCCGG	4567
	CCGGCGAGAGACCTGTC	4568
Sulfonylurea Resistance	GCGGGTTAGCCGACGCGATGCTTGACAGTGTTCCTCTCGTCGCC ATCACAGGACAGG	4569
ALS 3 Brassica napus Pro179Gln CCT-CAA	GACCTCGTTACCTCAACGATTGGCGTCTCTTTGGAACGCGTCAGTA CCGATCATCCGGCGTTGGACCTGTCCTGT	4570
001-074	ACAGGTCCAACGCCGGA	457
	TCCGGCG <u>TT</u> GGACCTGT	457
Sulfonylurea Resistance ALS 3	GCGGGTTAGCCGACGCGATGCTTGACAGTGTTCCTCTCGCCCATCACAGGACAGGTCCAGCGCCGGATGATCGGTACTGACGCGTTCCCAAGAGACGCCCAATCGTTGAGGTAACGAGGTC	457
Brassica napus Pro179Gln CCT-CAG	GACCTCGTTACCTCAACGATTGGCGTCTCTTGGAACGCGTCAGTA CCGATCATCCGGCG <u>CT</u> GGACCTGTCCTGTGATGGCGACGAGAGG AACACTGTCAAGCATCGCGTCGGCTAACCCGC	457
	ACAGGTCCAGCGCCGGA	457
	TCCGGCGCTGGACCTGT	457
Imidazolinone Resistance ALS 3	GACCGTACCTGTTGGATGTCATCTGTCCGCACCAAGAACATGTGT TACCGATGATCCCAAATGGTGGCACTTTCAAAGATGTAATAACCG AAGGGGATGGTCGCACTAAGTACTGAGAGAT	457
Brassica napus Ser635Asn AGT-AAT	ATCTCTCAGTACTTAGTGCGACCATCCCCTTCGGTTATTACATCTT TGAAAGTGCCACCATTTGGGATCATCGGTAACACATGTTCTTGGT GCGGACAGATGACATCCAACAGGTACGGTC	457

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ II
	GATCCCAA <u>A</u> TGGTGGCA	4579
	TGCCACCATTTGGGATC	4580
Sulfonylurea Resistance ALS Oryza sativa	TCCGCGCTCGCCGACGCGCTGCTCGACTCCGATGGTCG CCATCACGGGCCAGGTCTCCCGCCGCATGATCGGCACCGACGC CTTCCAGGAGACGCCCATAGTCGAGGTCACCCGCT	4581
Pro171Ser CCC-TCC	AGCGGGTGACCTCGACTATGGGCGTCTCCTGGAAGGCGTCGGTG CCGATCATGCGGCGGGAGACCTGGCCCGTGATGGCGACCATCG GGACGGAGTCGAGCAGCGCGTCGGCGAGCGCGGA	
	GCCAGGTCTCCCGCCGC GCGGCGGAGACCTGGC	4583
Sulfonylurea	<u> </u>	4584
Resistance ALS	CCGCGCTCGCCGACGCGCTGCTCGACTCCGTCCCGATGGTCGC CATCACGGGCCAGGTCCAACGCCGCATGATCGGCACCGCC TTCCAGGAGACGCCCATAGTCGAGGTCACCCGCTC	4585
Oryza sativa Pro171Gln CCC-CAA	GAGCGGGTGACCTCGACTATGGGCGTCTCTCTGGAAGGCGTCGGT GCCGATCATGCGGCG <u>TT</u> GGACCTGGCCCGTGATGGCGACCATCG GGACGGAGTCGAGCAGCGCGCGGCGGG	4586
	CCAGGTCC <u>AA</u> CGCCGCA	4587
	TGCGGCGTTGGACCTGG	4588
Sulfonylurea Resistance ALS	CCGCGCTCGCCGACGCGCTGCTCGACTCCGATGGTCGC CATCACGGGCCAGGTCCAGCGCCGCATGATCGGCACCGCC TTCCAGGAGACGCCCATAGTCGAGGTCACCCGCTC	4589
Oryza sativa Pro171Gin CCC-CAG	GAGCGGGTGACCTCGACTATGGGCGTCTCCTGGAAGGCGTCGGT GCCGATCATGCGGCG <u>CT</u> GGACCTGGCCCGTGATGGCGACCATC GGGACGGAGTCGAGCAGCGCGTCGGCGAGCGCGG	4590
	CCAGGTCC <u>AG</u> CGCCGCA	4591
	TGCGGCG <u>CT</u> GGACCTGG	4592
midazolinone Resistance ALS	GGCCATACTTGTTGGATATCATCGTCCCGCACCAGGAGCATGTGC TGCCTATGATCCCAAATGGGGGCGCATTCAAGGACATGATCCTGG ATGGTGATGGCAGGACTGTGTATTAATCTAT	4593
Oryza sativa Ser627Asn AGT-AAT	ATAGATTAATACACAGTCCTGCCATCACCATCCAGGATCATGTCCT TGAATGCGCCCCCATTTGGGATCATAGGCAGCACATGCTCCTGGT GCGGGACGATGATATCCAACAAGTATGGCC	4594
	GATCCCAA <u>A</u> TGGGGGCG	4595
	CGCCCCATTTGGGATC	4596

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Sulfonylurea Resistance ALS	TCTGCGCTCGCAGACGCGTTGCTCGACTCCGTCCCCATGGTCGC CATCACGGGACAGGTGTCGCGACGCATGATTGGCACCGACGCCT TTCAGGAGACGCCCATCGTCGAGGTCACCCGCT	4597
Zea mays Pro165Ser CCG-TCG	AGCGGGTGACCTCGACGATGGGCGTCTCCTGAAAGGCGTCGGTG CCAATCATGCGTCGCGACACCTGTCCCGTGATGGCGACCATGGG GACGGAGTCGAGCAACGCGTCTGCGAGCGCAGA	4598
000-100	GACAGGTGTCGCGACGC	4599 4600
	GCGTCGCG <u>A</u> CACCTGTC	
Sulfonylurea Resistance ALS	CTGCGCTCGCAGACGCGTTGCTCGACTCCCCATGGTCGCC ATCACGGGACAGGTGCAGCGACGCCTT TCAGGAGACGCCCATCGTCGAGGTCACCCGCTC	4601
Zea mays Pro165Gln CCG-CAG	GAGCGGGTGACCTCGACGATGGCGTCTCTCTGAAAGGCGTCGGT GCCAATCATGCGTCGCTGCACCTGTCCCGTGATGGCGACCATGG GGACGGAGTCGAGCAACGCGTCTGCGAGCGCAG	4602
	ACAGGTGCAGCGCA	4603
	TGCGTCGC <u>T</u> GCACCTGT	4604
Imidazolinone Resistance ALS	GGCCGTACCTCTTGGATATAATCGTCCCGCACCAGGAGCATGTGT TGCCTATGATCCCTAATGGTGGGGCTTTCAAGGATATGATCCTGG ATGGTGATGGCAGGACTGTGTATTGATCCGT	4605
Zea mays Ser621Asn AGT-AAT	ACGGATCAATACACAGTCCTGCCATCACCATCCAGGATCATATCC TTGAAAGCCCCACCATTAGGGATCATAGGCAACACATGCTCCTGG TGCGGGACGATTATATCCAAGAGGTACGGCC	4606
	GATCCCTA <u>A</u> TGGTGGGG	4607
	CCCCACCATTAGGGATC	4608
Sulfonylurea Resistance ALS	AGTGGTCTCGCTGATGCAATGCTCGATAGTATCCCTCTCGTGGCG ATCACTGGTCAAGTCTCTCGTCGGATGATCGGTACCGATGCTTTC CAGGAAACTCCAATTGTTGAGGTAACAAGGT	4609
Gossypium hirsutum Pro186Ser CCT-TCT	ACCTTGTTACCTCAACAATTGGAGTTTCCTGGAAAGCATCGGTAC CGATCATCCGACGAGAGACTTGACCAGTGATCGCCACGAGAGGG ATACTATCGAGCATTGCATCAGCGAGACCACT	4610
	GTCAAGTCTCTCGTCGG	4611
·	CCGACGAGAG <u>A</u> CTTGAC	4612
Sulfonylurea Resistance ALS	GTGGTCTCGCTGATGCAATGCTCGATAGTATCCCTCTCGTGGCGA TCACTGGTCAAGTCCAACGTCGGATGATCGGTACCGATGCTTTCC AGGAAACTCCAATTGTTGAGGTAACAAGGTC	4613
Gossypium hirsutum Pro186Gln CCT-CAA	GACCTTGTTACCTCAACAATTGGAGTTTCCTGGAAAGCATCGGTA CCGATCATCCGACGTTGGACTTGACCAGTGATCGCCACGAGAGG GATACTATCGAGCATTGCATCAGCGAGACCAC	4614
	TCAAGTCC <u>AA</u> CGTCGGA	4615

Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID
	TCCGACG <u>TT</u> GGACTTGA	4616
Sulfonylurea Resistance ALS	GTGGTCTCGCTGATGCAATGCTCGATAGTATCCCTCTCGTGGCGA TCACTGGTCAAGTCCAGCGTCGGATGATCGGTACCGATGCTTTCC AGGAAACTCCAATTGTTGAGGTAACAAGGTC	4617
Gossypium hirsutum Pro186GIn CCT-CAG	GACCTTGTTACCTCAACAATTGGAGTTTCCTGGAAAGCATCGGTA CCGATCATCCGACG <u>CT</u> GGACTTGACCAGTGATCGCCACGAGAGG GATACTATCGAGCATTGCATCAGCGAGACCAC	4618
	TCAAGTCC <u>AG</u> CGTCGGA	4619
	TCCGACG <u>CT</u> GGACTTGA	4620
Imidazolinone Resistance ALS	GACCTTACTTGTTGGATGTGATTGTCCCACATCAAGAACATGTCCT GCCTATGATCCCCAATGGAGGGGCTTTCAAAGATGTGATCACAGA GGGTGATGGAAGAACACAATATTGACCTCA	4621
Gossypium hirsutum Ser642Asn AGT-AAT	TGAGGTCAATATTGTGTTCTTCCATCACCCTCTGTGATCACATCTT TGAAAGCCCCTCCATTGGGGATCATAGGCAGGACATGTTCTTGAT GTGGGACAATCACATCCAACAAGTAAGGTC	4622
•	GATCCCCA <u>A</u> TGGAGGGG	4623
	CCCCTCCA <u>T</u> TGGGGATC	4624
Sulfonylurea Resistance ALS	TCTGGTCTTGCTGATGCACTTCTTGACTCAGTCCCTCTTGTCGCC ATTACTGGGCAAGTT <u>T</u> CCCGGCGTATGATTGGTACTGATGCTTTTC AAGAGACTCCAATTGTTGAGGTAACTCGAT	4625
Amaranthus powellii Pro192Ser CCC-TCC	ATCGAGTTACCTCAACAATTGGAGTCTCTTGAAAAGCATCAGTACC AATCATACGCCGGGAAACTTGCCCAGTAATGGCGACAAGAGGGA CTGAGTCAAGAAGTGCATCAGCAAGACCAGA	4626
	GGCAAGTT <u>T</u> CCCGGCGT	4627
	ACGCCGGG <u>A</u> AACTTGCC	4628
Sulfonylurea Resistance ALS	CTGGTCTTGCTGATGCACTTCTTGACTCAGTCCCTCTTGTCGCCA TTACTGGGCAAGTTCAACGGCGTATGATTGGTACTGATGCTTTTC AAGAGACTCCAATTGTTGAGGTAACTCGATC	4629
Amaranthus powellii Pro192GIn CCC-CAA	GATCGAGTTACCTCAACAATTGGAGTCTCTTGAAAAGCATCAGTAC CAATCATACGCCG <u>TT</u> GAACTTGCCCAGTAATGGCGACAAGAGGGA CTGAGTCAAGAAGTGCATCAGCAAGACCAG	4630
	GCAAGTTC <u>AA</u> CGGCGTA	4631
	TACGCCG <u>TT</u> GAACTTGC	4632
Sulfonylurea Resistance ALS	CTGGTCTTGCTGATGCACTTCTTGACTCAGTCCCTCTTGTCGCCA TTACTGGGCAAGTTCAGCGGCGTATGATTGGTACTGATGCTTTTC AAGAGACTCCAATTGTTGAGGTAACTCGATC	4633
Amaranthus powellii Pro192Gln CCC-CAG	GATCGAGTTACCTCAACAATTGGAGTCTCTTGAAAAGCATCAGTAC CAATCATACGCCG <u>CT</u> GAACTTGCCCAGTAATGGCGACAAGAGGG ACTGAGTCAAGAAGTGCATCAGCAAGACCAG	4634

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
	GCAAGTTC <u>AG</u> CGGCGTA	4635
	TACGCCG <u>CT</u> GAACTTGC	4636
Imidazolinone Resistance ALS	GACCGTATCTGCTGGATGTAATCGTACCACATCAGGAGCATGTGC TGCCTATGATCCCTAACGGTGCCGCCTTCAAGGACACCATAACAG AGGGTGATGGAAGAAGAGGGCTTATTAGTTGGT	4637
Amaranthus powellii Ser652Asn AGC-AAC	ACCAACTAATAAGCCCTTCTTCCATCACCCTCTGTTATGGTGTCCT TGAAGGCGGCACCGTTAGGGATCATAGGCAGCACATGCTCCTGA TGTGGTACGATTACATCCAGCAGATACGGTC	4638
	GATCCCTA <u>A</u> CGGTGCCG	4639
	CGGCACCGTTAGGGATC	4640

Table 12

<u>Genome-Altering Oligos Conferring Porphyric Herbicide Resistance</u>

5	Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID
•	Porphyric Herbicide Resistant PPO	TCTTGCGCCCTCTTTCTGAATCTGCTGCAAATGCACTCTCAAAACT ATATTACCCACCAATGGCAGCAGTATCTATCTCGTACCCGAAAGA AGCAATCCGAACAGAATGTTTGATAGATGG	4641
10	Arabidopsis thaliana Val365Met GTT-ATG	CCATCTATCAAACATTCTGTTCGGATTGCTTCTTTCGGGTACGAGA TAGATACTGCTGCCATTGGTGGGTAATATAGTTTTGAGAGTGCATT TGCAGCAGATTCAGAAAGAGGGCGCAAGA	4642
		CCCACCA <u>A</u> T <u>G</u> GCAGCAG	4643
		CTGCTGC <u>C</u> A <u>T</u> TGGTGGG	4644
	Porphyric Herbicide Resistant PPO	TATTACGTCCTCTTTCGGTTGCCGCAGCAGATGCACTTTCAAATTT CTACTATCCCCCAATGGGAGCAGTCACAATTTCATATCCTCAAGAA GCTATTCGTGATGAGCGTCTGGTTGATGG	4645
15	Nicotiana tabacum Val376Met GTT-ATG	CCATCAACCAGACGCTCATCACGAATAGCTTCTTGAGGATATGAA ATTGTGACTGCTCCCATTGGGGGGATAGTAGAAATTTGAAAGTGCA TCTGCTGCGGCAACCGAAAGAGGACGTAATA	4646
		TCCCCAATGGGAGCAG	4647
		CTGCTCC <u>C</u> A <u>T</u> TGGGGGA	4648
20	Porphyric Herbicide Resistant PPO	TGTTGCGTCCGCTTTCGTTGGGTGCAGCAGATGCATTGTCAAAAT TTTATTATCCTCCGATGGCAGCTGTATCAATTTCATATCCAAAAGA CGCAATTCGTGCTGACCGGCTGATTGATGG	4649
	Cichorium intybus Val383Met GTT-ATG	CCATCAATCAGCCGGTCAGCACGAATTGCGTCTTTTGGATATGAA ATTGATACAGCTGCCATCGGAGGATAATAAAATTTTGACAATGCAT CTGCTGCACCCAACGAAAGCGGACGCAACA	4650
		TCCTCCGATGGCAGCTG	4651
		CAGCTGC <u>C</u> A <u>T</u> CGGAGGA	4652
25	Porphyric Herbicide Resistant PPO	TCCTTCGTCCACTTTCAGATGTCGCCGCAGAATCTCTTTCAAAATT TCATTATCCACCAATGCCAGCTGTGTCACTTTCCTATCCTAAAGAA GCAATTAGATCAGAGTGCTTGATTGACGG	4653
	Spinacia oleracea Val390Met GTT-ATG	CCGTCAATCAAGCACTCTGATCTAATTGCTTCTTTAGGATAGGAAA GTGACACAGCTGCCATTGGTGGATAATGAAATTTTGAAAGAGATTC TGCGGCGACATCTGAAAGTGGACGAAGGA	4654
		TCCACCAATGGCAGCTG	4655
		CAGCTGC <u>C</u> A <u>T</u> TGGTGGA	4656

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Porphyric Herbicide Resistant PPO	TTTTGCGTCCACTTTCAAGCGATGCTGCAGATGCTCTATCAAGATT CTATTATCCACCGATGGCTGCTGTAACTGTTTCGTATCCAAAGGAA GCAATTAGAAAAGAATGCTTAATTGATGG	4657
Zea mays Val363Met GTT-ATG	CCATCAATTAAGCATTCTTTTCTAATTGCTTCCTTTGGATACGAAAC AGTTACAGCAGCCATCGGTGGATAATAGAATCTTGATAGAGCATC TGCAGCATCGCTTGAAAGTGGACGCAAAA	4658
	TCCACCGATGGCTGCTG	4659
	CAGCAGC <u>C</u> A <u>T</u> CGGTGGA	4660
Porphyric Herbicide Resistant PPO	TCTTGCGGCCACTTTCAAGTGATGCAGCAGATGCTCTGTCAATATT CTATTATCCACCAATGGCTGCTGTAACTGTTTCATATCCAAAAGAA GCAATTAGAAAAGAATGCTTAATTGACGG	4661
Oryza sativa Val364Met GTT-ATG	CCGTCAATTAAGCATTCTTTTCTAATTGCTTCTTTTTGGATATGAAAC AGTTACAGCAGCCATTGGTGGATAATAGAATATTGACAGAGCATCT GCTGCATCACTTGAAAGTGGCCGCAAGA	4662
	TCCACCAATGGCTGCTG	4663
	CAGCAGCCA <u>T</u> TGGTGGA	4664
Porphyric Herbicide Resistant PPO	CTGGTCAAGGAGCAGGCGCCGCCGCCGCGAGGCCCTGGGCT CCTTCGACTACCCGCCGATGGGCGCCGTGACGCTGTCGTACCCG CTGAGCGCCGTGCGGGAGGAGCGCAAGGCCTCGG	4665
Chlamydomonas reinhardtii Val389Met	CCGAGGCCTTGCGCTCCTCCCGCACGGCGCTCAGCGGTACGA CAGCGTCACGGCGCCCATCGGCGGGTAGTCGAAGGAGCCCAGG GCCTCGGCGGCGGCGCGCCTGCTCCTTGACCAG	4666
GTG-ATG	ACCCGCCGATGGGCGCC	4667
	GGCGCCCA <u>T</u> CGGCGGGT	4668

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Table 13
Genome-Altering Oligos Conferring Triazine Resistance

Phenotype, Gene, SEGID **Altering Oligos** Plant & Targeted NO: Alteration 4669 Triazine Resistant AAACTTACAACATTGTAGCTGCTCACGGTTATTTTGGCCGATTGAT  ${\tt TTTCCAATATGCTA} \underline{{\tt CTTTCAACAATTCTCGTTCTTTACATTTCTTCTT}$ D1 Protein Arabidopsis thaliana AGCGGCTTGGCCGGTAGTAGGTATTTG CAAATACCTACTACCGGCCAAGCCGCTAAGAAGAAATGTAAAGAA 4670 Ser264Thr CGAGAATTGTTGAAAGTAGCATATTGGAAAATCAATCGGCCAAAAT AGT-ACT AACCGTGAGCAGCTACAATGTTGTAAGTTT

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID No:
	ATATGCTA <u>C</u> TTTCAACA	4671
1	TGTTGAAAGTAGCATAT	4672
Triazine Resistant	AAACTTATAACATCGTAGCCGCTCATGGTTATTTTGGCCGATTGAT	4673
D1 Protein Nicotiana tabacum Ser264Thr AGT-ACT	CTTCCAATATGCTACTTTCAACAACTCTCGTTCGTTACACTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4075
	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAGTGTAACGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAA TAACCATGAGCGGCTACGATGTTATAAGTTT	4674
	ATATGCTACTTTCAACA	4675
	TGTTGAAA <u>G</u> TAGCATAT	4676
Triazine Resistant D1 Protein Populus deltoides	AAACTTATAATATCGTAGCCGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTTAACAACTCTCGCTCTTTACATTTCTTCT TAGCTGCTTGGCCTGTAGTAGGTATCTG	4677
Ser264Thr AGT-ACT	CAGATACCTACTACAGGCCAAGCAGCTAAGAAGAAATGTAAAGAG CGAGAGTTGTTAAAAGTAGCATATTGGAAGATCAATCGGCCAAAAT AACCATGAGCGGCTACGATATTATAAGTTT	4678
	ATATGCTA <u>C</u> TTTTAACA	4679
Tion D. I. I.	TGTTAAAAGTAGCATAT	4680
Triazine Resistant D1 Protein Petunia x hybrida Ser264Thr	AAACTTATAATATCGTAGCCGCTCATGGTTATTTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAACTCTCGTTCGTTACACTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4681
AGT-ACT	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAGTGTAACGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAA TAACCATGAGCGGCTACGATATTATAAGTTT	4682
	ATATGCTA <u>C</u> TTTCAACA	4683
	TGTTGAAA <u>G</u> TAGCATAT	4684
Triazine Resistant D1 Protein Magnolia pyramidata Ser264Thr AGT-ACT	AAACTTATAATATCGTAGCTGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAATTCTCGTTCTTTACATTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4685
	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAAT AACCATGAGCAGCTACGATATTATAAGTTT	4686
	ATATGCTACTTTCAACA	4687
T	TGTTGAAA <u>G</u> TAGCATAT	4688
Triazine Resistant D1 Protein Medicago sativa	AAACCTATAATATTGTAGCAGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAACTCTCGTTCTTTACATTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4689
Ser264Thr AGT-ACT	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAATGTAAAGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAA TAACCATGAGCTGCTACAATATTATAGGTTT	4690
	ATATGCTA <u>C</u> TTTCAACA	4691

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
	TGTTGAAA <u>G</u> TAGCATAT	4692
Triazine Resistant D1 Protein Glycine max Ser264Thr AGT-ACT	AAACCTATAATATTGTAGCTGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCAACTTTCAACAATTCTCGTTCTTTACATTTCTTCT TAGCTGCTTGGCCTGTAGTAGGTATTTG	4693
	CAAATACCTACTACAGGCCAAGCAGCTAAGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTTGCATATTGGAAGATCAATCGGCCAAAAT AACCATGAGCAGCTACAATATTATAGGTTT	4694
	ATATGCAA <u>C</u> TTTCAACA	4695
	TGTTGAAA <u>G</u> TTGCATAT	4696
Triazine Resistant D1 Protein Brassica napus Gly264Thr GGT-ACT	AAACTTACAACATTGTAGCTGCTCACGGTTATTTTGGCCGATTGAT CTTCCAATATGCT <u>AC</u> TTTCAACAATTCTCGTTCTTTACATTTCTTCT TAGCGGCTTGGCCGGTAGTAGGTATTTG	4697
	CAAATACCTACTACCGGCCAAGCCGCTAAGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAAT AACCGTGAGCAGCTACAATGTTGTAAGTTT	4698
	ATATGCT <u>AC</u> TTTCAACA	4699
	TGTTGAAA <u>GT</u> AGCATAT	4700
Triazine Resistant D1 Protein Onyza sativa Ser264Thr AGT-ACT	AAACTTATAATATTGTGGCCGCTCATGGTTATTTTGGCCGATTAAT CTTCCAATATGCTACTTTTAACAACTCTCGTTCTTTACACTTCTTCT TGGCTGCTTGGCCTGTAGTAGGGATTTG	4701
	CAAATCCCTACTACAGGCCAAGCAGCCAAGAAGAAGTGTAAAGAA CGAGAGTTGTTAAAAGTAGCATATTGGAAGATTAATCGGCCAAAAT AACCATGAGCGGCCACAATATTATAAGTTT	4702
•	ATATGCTA <u>C</u> TTTTAACA	4703
	TGTTAAAAGTAGCATAT	4704
Triazine Resistant D1 Protein Zea mays	AGACTTATAATATTGTGGCTGCTCACGGTTATTTTGGTCGATTAAT CTTCCAATATGCTACTTTCAACAATTCTCGTTCTTTACACTTCTTCT TGGCTGCTTGGCCTGTAGTAGGGATCTG	4705
Ser264Thr AGT-ACT	CAGATCCCTACTACAGGCCAAGCAGCCAAGAAGAAGTGTAAAGAA CGAGAATTGTTGAAAGTAGCATATTGGAAGATTAATCGACCAAAAT AACCGTGAGCAGCCACAATATTATAAGTCT	4706
	ATATGCTACTTTCAACA	4707
	TGTTGAAAGTAGCATAT	4708
Triazine Resistant D1 Protein Arabidopsis thaliana	AAACTTACAACATTGTAGCTGCTCACGGTTATTTTGGCCGATTGAT TTTCCAATATGCTACTTTCAACAATTCTCGTTCTTTACATTTCTTCTT AGCGGCTTGGCCGGTAGTAGGTATTTG	4709
Ser264Thr AGT-ACT	CAAATACCTACTACCGGCCAAGCCGCTAAGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTAGCATATTGGAAAATCAATCGGCCAAAAT AACCGTGAGCAGCTACAATGTTGTAAGTTT	4710
	ATATGCTA <u>C</u> TTTCAACA	4711
	TGTTGAAAGTAGCATAT	4712

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Triazine Resistant D1 Protein Nicotiana tabacum Ser264Thr AGT-ACT	AAACTTATAACATCGTAGCCGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAACTCTCGTTCGTTACACTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4713
	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAGTGTAACGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAA TAACCATGAGCGGCTACGATGTTATAAGTTT	4714
	ATATGCTA <u>C</u> TTTCAACA	4715
	TGTTGAAA <u>G</u> TAGCATAT	4716
Triazine Resistant D1 Protein Populus deltoides Ser264Thr AGT-ACT	AAACTTATAATATCGTAGCCGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTTAACAACTCTCGCTCTTTACATTTCTTCT TAGCTGCTTGGCCTGTAGTAGGTATCTG	4717
	CAGATACCTACTACAGGCCAAGCAGCTAAGAAGAAATGTAAAGAG CGAGAGTTGTTAAAAGTAGCATATTGGAAGATCAATCGGCCAAAAT AACCATGAGCGGCTACGATATTATAAGTTT	4718
	ATATGCTA <u>C</u> TTTTAACA	4719
	TGTTAAAAGTAGCATAT	4720
Triazine Resistant D1 Protein Petunia x hybrida Ser264Thr AGT-ACT	AAACTTATAATATCGTAGCCGCTCATGGTTATTTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAACTCTCGTTCGTTACACTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4721
	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAGTGTAACGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAA TAACCATGAGCGGCTACGATATTATAAGTTT	4722
	ATATGCTA <u>C</u> TTTCAACA	4723
	TGTTGAAA <u>G</u> TAGCATAT	4724
Triazine Resistant D1 Protein Magnolia pyramidata	AAACTTATAATATCGTAGCTGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAATTCTCGTTCTTTACATTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4725
Ser264Thr AGT-ACT	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAAT AACCATGAGCAGCTACGATATTATAAGTTT	4726
	ATATGCTA <u>C</u> TTTCAACA	4727
	TGTTGAAAGTAGCATAT	4728
Triazine Resistant D1 Protein Medicago sativa Ser264Thr AGT-ACT	AAACCTATAATATTGTAGCAGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAACTCTCGTTCTTTACATTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4729
	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAATGTAAAGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAA TAACCATGAGCTGCTACAATATTATAGGTTT	4730
	ATATGCTA <u>C</u> TTTCAACA	4731
	TGTTGAAA <u>G</u> TAGCATAT	4732

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID No:
Triazine Resistant D1 Protein Glycine max Ser264Thr AGT-ACT	AAACCTATAATATTGTAGCTGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCAACTTTCAACAATTCTCGTTCTTTACATTTCTTCT TAGCTGCTTGGCCTGTAGTAGGTATTTG	4733
	CAAATACCTACTACAGGCCAAGCAGCTAAGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTTGCATATTGGAAGATCAATCGGCCAAAAT AACCATGAGCAGCTACAATATTATAGGTTT	4734
	ATATGCAACA .	4735
	TGTTGAAA <u>G</u> TTGCATAT	4736
Triazine Resistant D1 Protein Brassica napus	AAACTTACAACATTGTAGCTGCTCACGGTTATTTTGGCCGATTGAT CTTCCAATATGCT <u>AC</u> TTTCAACAATTCTCGTTCTTTACATTTCTTCT TAGCGGCTTGGCCGGTAGTAGGTATTTG	4737
Gly264Thr GGT-ACT	CAAATACCTACTACCGGCCAAGCCGCTAAGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAAT AACCGTGAGCAGCTACAATGTTGTAAGTTT	4738
	ATATGCTACTTTCAACA	4739
	TGTTGAAA <u>GT</u> AGCATAT	4740
Triazine Resistant D1 Protein Oryza sativa	AAACTTATAATATTGTGGCCGCTCATGGTTATTTTGGCCGATTAAT CTTCCAATATGCTACTTTTAACAACTCTCGTTCTTTACACTTCTTCT TGGCTGCTTGGCCTGTAGTAGGGATTTG	4741
Ser264Thr AGT-ACT	CAAATCCCTACTACAGGCCAAGCAGCCAAGAAGAAGTGTAAAGAA CGAGAGTTGTTAAAAGTAGCATATTGGAAGATTAATCGGCCAAAAT AACCATGAGCGGCCACAATATTATAAGTTT	4742
	ATATGCTA <u>C</u> TTTTAACA	4743
	TGTTAAAA <u>G</u> TAGCATAT	4744
Triazine Resistant D1 Protein Zea mays	AGACTTATAATATTGTGGCTGCTCACGGTTATTTTGGTCGATTAAT CTTCCAATATGCTACTTTCAACAATTCTCGTTCTTTACACTTCTTCT TGGCTGCTTGGCCTGTAGTAGGGATCTG	4745
Ser264Thr AGT-ACT	CAGATCCCTACTACAGGCCAAGCAGCCAAGAAGAAGAACCGAGAATTGTTGAAAGTAGCATATTGGAAGATTAATCGACCAAAATAACCGTGAGCAGCCACAATATTATAAGTCT	4746
	ATATGCTA <u>C</u> TTTCAACA	4747
	TGTTGAAAGTAGCATAT	4748
Triazine Resistant D1 Protein Arabidopsis thaliana Ser264Thr AGT-ACT	AAACTTACAACATTGTAGCTGCTCACGGTTATTTTGGCCGATTGAT TTTCCAATATGCTACTTTCAACAATTCTCGTTCTTTACATTTCTTCTT AGCGGCTTGGCCGGTAGTAGGTATTTG	4749
	CAAATACCTACTACCGGCCAAGCCGCTAAGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTAGCATATTGGAAAATCAATCGGCCAAAAT AACCGTGAGCAGCTACAATGTTGTAAGTTT	4750
	ATATGCTA <u>C</u> TTTCAACA	4751
	TGTTGAAA <b>G</b> TAGCATAT	4752

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID
Triazine Resistant D1 Protein Picea abies Ser264Thr	AAACCTACAATATTGTGGCTGCTCACGGTTATTTCGGCCGATTGAT CTTCCAGTATGCTACTTTCAACAACTCCCGTTCTTTACATTTCTTCT TAGCTGCTTGGCCCGTAGCAGGTATCTG	
AGT-ACT	CAGATACCTGCTACGGGCCAAGCAGCTAAGAAGAAATGTAAAGAA CGGGAGTTGTTGAAAGTAGCATACTGGAAGATCAATCGGCCGAAA TAACCGTGAGCAGCCACAATATTGTAGGTTT	4754
	GTATGCTACTTCAACA	4755
Triazina Pocietant	TGTTGAAAGTAGTAGTAGTAGTAGTAGTAGTAGTAGTAGT	4756
Triazine Resistant D1 Protein Vicia faba Ser264Thr AGT-ACT	AAACCTATAATATTGTAGCTGCTCACGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAATTCTCGCTCTTTACATTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4757
	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAATGTAAAGAG CGAGAATTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAAT AACCGTGAGCAGCTACAATATTATAGGTTT	4758
	ATATGCTA <u>C</u> TTTCAACA	4759
<del></del>	TGTTGAAA <u>G</u> TAGCATAT	4760
Triazine Resistant D1 Protein Hordeum vulgare	AGACTTATAATATTGTGGCTGCTCATGGTTATTTTGGCCGATTAAT CTTCCAATATGCTACTTTCAACAACTCTCGTTCTTTACACTTCTTCT TGGCTGCTTGGCCTGTAGTAGGAATCTG	4761
Ser264Thr AGT-ACT	CAGATTCCTACTACAGGCCAAGCAGCCAAGAAGAAGTGTAAAGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATTAATCGGCCAAAAT AACCATGAGCAGCCACAATATTATAAGTCT	4762
	ATATGCTACTTTCAACA	4763
	TGTTGAAAGTAGCATAT	4764
Triazine Resistant D1 Protein Triticum aestivum	AAACTTATAATATTGTGGCTGCTCATGGTTATTTTGGCCGATTAAT CTTCCAATATGCTACTTCAACAACTCTCGTTCTTTACACTTCTTCT TGGCTGCTTGGCCTGTAGTAGGAATCTG	4765
Ser264Thr AGT-ACT	CAGATTCCTACTACAGGCCAAGCAGCCAAGAAGAAGTGTAAAGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATTAATCGGCCAAAAT AACCATGAGCAGCCACAATATTATAAGTTT	4766
	ATATGCTACTTTCAACA	4767
Control Decision	TGTTGAAA <u>G</u> TAGCATAT	4768
riazine Resistant 01 Protein /igna unguiculata	AAACTTATAATATTGTAGCTGCTCATGGTTATTTTGGCCGATTAATC TTCCAATATGCAACTTTCAACAATTCTCGTTCTTTACATTTCTTCCT AGCTGCTTGGCCTGTAGTAGTAGGTATTTG	4769
IGI-ACI	CAAATACCTACTACAGGCCAAGCAGCTAGGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTTGCATATTGGAAGATTAATCGGCCAAAAT AACCATGAGCAGCTACAATATTATAAGTTT	4770
	ATATGCAA <u>C</u> TTTCAACA	4771
	TGTTGAAAGTTGCATAT	4772

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Triazine Resistant D1 Protein Lotus japonicus	AAACCTATAATATTGTAGCTGCTCACGGTTATTTTGGCCGATTGAT CTTCCAATATGCAACTTTCAACAACTCTCGTTCTTTACACTTCTTCT TAGCTGCTTGGCCTGTTGTAGGTATCTG	4773
Ser264Thr AGT-ACT	CAGATACCTACAACAGGCCAAGCAGCTAAGAAGAAGTGTAAAGAA CGAGAGTTGTTGAAAGTTGCATATTGGAAGATCAATCGGCCAAAAT AACCGTGAGCAGCTACAATATTATAGGTTT	4774
	ATATGCAACTTTCAACA	4775
	TGTTGAAAGTTGCATAT	4776
Triazine Resistant D1 Protein Sinapis alba	AAACTTACAACATTGTAGCTGCTCACGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAATTCTCGTTCTTTACATTTCTTCT TAGCGGCTTGGCCGGTAGTAGGTATTTG	4777
Ser264Thr AGT-ACT	CAAATACCTACTACCGGCCAAGCCGCTAAGAAGAAATGTAAAGAA CGAGAATTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAAT AACCGTGAGCAGCTACAATGTTGTAAGTTT	4778
	ATATGCTACTTTCAACA	4779
	TGTTGAAAGTAGCATAT	4780
Triazine Resistant D1 Protein	AAACCTATAATATTGTAGCTGCTCACGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAATTCTCGCTCTTTACATTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4781
Pisum sativum Ser264Thr AGT-ACT	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAATGTAAAGAG CGAGAATTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAAT AACCGTGAGCAGCTACAATATTATAGGTTT	4782
	ATATGCTACTTTCAACA	4783
	TGTTGAAAGTAGCATAT	4784
Triazine Resistant D1 Protein Spinacia oleracea	AAACTTATAATATCGTAGCTGCTCATGGTTATTTTGGTCGATTGAT CTTCCAATATGCTACTTTCAACAACTCTCGTTCTTTACACTTCTTCT TAGCTGCTTGGCCTGTAGTAGGTATTTG	4785
Ser264Thr AGT-ACT	CAAATACCTACTACAGGCCAAGCAGCTAAGAAGAAGTGTAAAGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGACCAAAAT AACCATGAGCAGCTACGATATTATAAGTTT	4786
	ATATGCTACTTTCAACA	4787
	TGTTGAAAGTAGCATAT	4788
Triazine Resistant D1 Protein Nicotiana debneyi	AAACTTATAACATCGTAGCCGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAACTCTCGTTCGTTACACTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4789
Ser264Thr AGT-ACT	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAGTGTAACGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAA TAACCATGAGCGGCTACGATGTTATAAGTTT	4790
	ATATGCTACTTTCAACA	4791
	TGTTGAAAGTAGCATAT	4792

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Triazine Resistant D1 Protein Solanum nigrum	AAACITATAATATCGTAGCCGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTTCAACAACTCTCGTTCGTTACACTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4793
Ser264Thr AGT-ACT	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAGTGTAACGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAA TAACCATGAGCGGCTACGATATTATAAGTTT	4794
	ATATGCTA <u>C</u> TTTCAACA	4795
	TGTTGAAA <u>G</u> TAGCATAT	4796
Triazine Resistant D1 Protein Nicotiana	AAACTTATAACATCGTAGCCGCTCATGGTTATTTTGGCCGATTGAT CTTCCAATATGCTACTTCAACAACTCTCGTTCGTTACACTTCTTCC TAGCTGCTTGGCCTGTAGTAGGTATCTG	4797
<i>plumbaginifolia</i> Ser264Thr AGT-ACT	CAGATACCTACTACAGGCCAAGCAGCTAGGAAGAAGTGTAACGAA CGAGAGTTGTTGAAAGTAGCATATTGGAAGATCAATCGGCCAAAA TAACCATGAGCGGCTACGATGTTATAAGTTT	4798
	ATATGCTA <u>C</u> TTTCAACA	4799
	TGTTGAAA <u>G</u> TAGCATAT	4800

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## Example 6 Engineering male- or female-sterile plants

Flower development in distantly related dicot plant species is increasingly better understood and appears to be regulated by a family of genes which encode regulatory proteins. These genes include, for example, AGAMOUS (AG), APETALA1 (AP1), and APETALA3 (AP3) and PISTILLATA (PI) in Arabidopsis thaliana, and DEFICIENS A (DEFA), GLOBOSA (GLO), SQUAMOSA (SQUA), and PLENA (PLE) in Antimhinum majus. Genetic studies have shown that the DEFA, GLO and AP3 genes are essential for petal and stamen development. Sequence analysis of these genes revealed that the gene products contain a conserved MADS box region, a DNA-binding domain. Using these clones as probes, MADS box genes have also been isolated from other species including tomato, tobacco, petunia, Brassica napus, and maize.

Altering the expression of these genes results in altered floral morphology. For example, mutations in AP3 and PI result in male-sterile flowers because petals develop in place of stamens.

The attached tables disclose exemplary oligonucleotide base sequences which can be used to generate site-specific mutations that confer altered floral structures in plants.

Table 14
Oligonucleotides to produce male-sterile plants

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ.ID NO:
Male-sterile AP3 Arabidopsis thaliana Arg3Term AGA-TGA	TTGTCCTCTCCACCAAATCTCTTCAACAAAAAGATTAAACAAAGAGA GAAGAATATGGCGTGAGGGAAGATCCAGATCAAGAGGATAGAGAA CCAGACAAACAGACAAGTGACGTATTCAA	4801
	TTGAATACGTCACTTGTCTGTTTGTCTGGTTCTCTATCCTCTTGATC TGGATCTTCCCTCACGCCATATTCTTCTCTCTTTGTTTAATCTTTTT GTTGAAGAGATTTGGTGGAGAGACAA	4802
	ATATGGCGTGAGGGAAG	4803
	CTTCCCTCACGCCATAT	4804
Male-sterile AP3 <i>Arabidopsis thaliana</i>	TCTCCACCAAATCTCTTCAACAAAAAGATTAAACAAAGAGAGAG	4805
Lys5Term AAG-TAG	TTCTCTTTGAATACGTCACTTGTCTGTTTGTCTGGTTCTCTATCCTC TTGATCTGGATCTACCCTCTCGCCATATTCTTCTCTCTTTGTTTAAT CTTTTTGTTGAAGAGATTTGGTGGAGA	4806
	CGAGAGGTAGATCCAG	4807

	CTGGATCT <u>A</u> CCCTCTCG	4808
Male-sterile AP3 Arabidopsis thaliana Gln7Term	CCAAATCTCTTCAACAAAAAGATTAAACAAAGAGAGAAGAATATGG CGAGAGGGAAGATCTAGATCAAGAGGATAGAGAACCAGACAAACA GACAAGTGACGTATTCAAAGAGAAGGAATG	
CAG-TAG	CATTCCTTCTCTTGAATACGTCACTTGTCTGTTTGTCTGGTTCTCT ATCCTCTTGATCTAGATCTTCCCTCTCGCCATATTCTTCTCTCTTTG TTTAATCTTTTTGTTGAAGAGATTTGG	<u> </u>
	GGAAGATC <u>T</u> AGATCAAG CTTGATCT <u>A</u> GATCTTCC	4811
		4812
Male-sterile AP3 Arabidopsis thaliana	CTCTTCAACAAAAAGATTAAACAAAGAGAGAAGAATATGGCGAGAG GGAAGATCCAGATC <u>T</u> AGAGGATAGAGAACCAGACAAG TGACGTATTCAAAGAGAAGGAATGGTTTAT	4813
Lys9Term AAG-TAG	ATAAACCATTCCTTCTCTTTGAATACGTCACTTGTCTGTTTGTCTGG TTCTCTATCCTCTAGATCTGGATCTTCCCTCTCGCCATATTCTTCTC TCTTTGTTTAATCTTTTTGTTGAAGAG	4814
	TCCAGATC <u>T</u> AGAGGATA	4815
	TATCCTCTAGATCTGGA	4816
Male-sterile AP3 <i>Brassica oleracea</i> Lys23Term	AGAGGGAAGATCCAGATCAAGAGGGATAGAGAACCAGACCAACCGA CAAGTGACGTATTCTTAGAGAAGAAATGGTTTGTTCAAGAAAGCTC ACGAGCTTACAGTTTTATGTGATGCTAGGG	4817
AAG-TAG	CCCTAGCATCACATAAAACTGTAAGCTCGTGAGCTTTCTTGAACAA ACCATTTCTTCTAAGAATACGTCACTTGTCGGTTGGTCTGGTTC TCTATCCTCTTGATCTGGATCTTCCCTCT	<del>4</del> 818
	CGTATTCT <u>T</u> AGAGAAGA	4819
	TCTTCTCT <u>A</u> AGAATACG	4820
Male-sterile AP3 Brassica oleracea	GGGAAGATCCAGATCAAGAGGGATAGAGAACCAGACCAACCGACAA GTGACGTATTCTAAGTGAAGAAATGGTTTGTTCAAGAAAGCTCACG AGCTTACAGTTTTATGTGATGCTAGGGTTT	4821
Arg24Term AGA-TGA	AAACCCTAGCATCACATAAAACTGTAAGCTCGTGAGCTTTCTTGAA CAAACCATTTCTTCACTTAGAATACGTCACTTGTCGGTTGGTCTGG TTCTCTATCCTCTTGATCTGGATCTTCCC	4822
	ATTCTAAG <u>T</u> GAAGAAAT	4823
	ATTTCTTC <u>A</u> CTTAGAAT	4824
lale-sterile P3 trassica oleracea	AAGATCCAGATCAAGAGGATAGAGAACCAGACCAACCGACAAGTG ACGTATTCTAAGAGATGAAATGGTTTGTTCAAGAAAGCTCACGAGC TTACAGTTTTATGTGATGCTAGGGTTTCGA	4825
rg25Term GA-TGA	TCGAAACCCTAGCATCACATAAAACTGTAAGCTCGTGAGCTTTCTT GAACAAACCATTTCATCTTTAGAATACGTCACTTGTCGGTTGGTC TGGTTCTCTATCCTCTTGATCTGGATCTT	4826
]	CTAAGAGA <u>T</u> GAAATGGT	4827
	ACCATITC <u>A</u> TCTCTTAG	4828

المام مامام	TCAAGAGGATAGAGAACCAGACCAACCGACAAGTGACGTATTCTA	4829
Male-sterile AP3	AGAGAAGAATGGTTAGTTCAAGAAAGCTCACGAGCTTACAGTTTT	
APS Brassica oleracea	ATGTGATGCTAGGGTTTCGATTATCATGTT	
Leu28Term	AACATGATAATCGAAACCCTAGCATCACATAAAACTGTAAGCTCGT	4830
TTG-TAG	GAGCTITCTTGAACTAACCATTTCTTCTCTTAGAATACGTCACTTGT	
I I G-I AG	CGGTTGGTCTCGTCTCTCTCTCTGA	
	AAATGGTTAGTTCAAGA	4831
	TCTTGAACTAACCATTT	4832
Male-sterile	GGCTCGAGGGAAGATCCAGATTAAGAGGATAGAGAACCAAACAAA	4833
AP3	CAGGCAGGTCACCTAGTCCAAGAGAAAATGGTTTGTTCAAGAA	
Brassica napus	AGCACACGAGCTCTCTGTTCTCTGTGATGCT	
Tyr21Term	AGCATCACAGAGAACAGAGGCTCGTGTGCTTTCTTGAACAAACC	4834
TAC-TAG	ATTTCTTCTCTTGGACTAGGTGACCTGCCTGTTTGTTTGGTTCTCTA	
	TCCTCTTAATCTGGATCTTCCCTCGAGCC	
	GTCACCTAGTCCAAGAG	4835
	CTCTTGGA <u>C</u> TAGGTGAC	4836
Male-sterile	CGAGGGAAGATCCAGATTAAGAGGATAGAGAACCAAACAACAGG	4837
AP3	CAGGTCACCTACTCCTAGAGAAGAAATGGTTTGTTCAAGAAAGCAC	
Brassica napus	ACGAGCTCTCTGTTCTCTGTGATGCTAAAG	
Lys23Term	CTTTAGCATCACAGAGAACAGAGAGCTCGTGTGCTTTCTTGAACAA	4838
AAG-TAG	ACCATTTCTTCTCTAGGAGTAGGTGACCTGCCTGTTTGTT	
	TCTATCCTCTTAATCTGGATCTTCCCTCG	
	CCTACTCC <u>T</u> AGAGAAGA	4839
•	TCTTCTCTAGGAGTAGG	4840
Male-sterile	GGGAAGATCCAGATTAAGAGGATAGAGAACCAAACAAACA	4841
AP3	GTCACCTACTCCAAGTGAAGAAATGGTTTGTTCAAGAAAGCACACG	-
Brassica napus	AGCTCTCTGTTCTCTGTGATGCTAAAGTTT	
Arg24Term	AAACTTTAGCATCACAGAGAACAGAGAGCTCGTGTGCTTTCTTGAA	4842
AGA-TGA	CAAACCATTTCTTCACTTGGAGTAGGTGACCTGCCTGTTTGTT	
	TTCTCTATCCTCTTAATCTGGATCTTCCC	
	ACTCCAAG <u>T</u> GAAGAAAT	4843
	ATTTCTTCACTTGGAGT	4844
Male-sterile	AAGATCCAGATTAAGAGGATAGAGAACCAAACAAACAGGCAGG	4845
AP3	ACCTACTCCAAGAGATGAAATGGTTTGTTCAAGAAAGCACACGAGC	
Brassica napus	TCTCTGTTCTCTGTGATGCTAAAGTTTCCA	40.40
Arg25Term AGA-TGA	TGGAAACTTTAGCATCACAGAGAACAGAGAGCTCGTGTGCTTTCTT	4846
	GAACAAACCATTTCATCTCTTGGAGTAGGTGACCTGCCTG	
	TGGTTCTCTATCCTCTTAATCTGGATCTT	4047
	CCAAGAGA <u>T</u> GAAATGGT	4847
	ACCATTTCATCTCTTGG	4848

	Male-sterile	GGAGAGAAAGGAAAGCTGGAAGAAGAAACAAGAGCAGTAGTGGT	4849
	DEFA	AGTGGTTCGATGGCTTGAGGGAAGATCCAGATTAAGAGGATAGAG	7043
	Antimhinum majus	AACCAAACAAACAGGCAGGTCACCTACTCCA	1
_	Arg3Term	TGGAGTAGGTGACCTGCCTGTTTGTTTGGTTCTCTATCCTCTTAAT	4850
5	CGA-TGA	CTGGATCTTCCCTCAAGCCATCGAACCACTACCACTACTGCTCTTG	
		TITTCTTCCAGCTTTCCTTTCTCCC	1
		CGATGGCT <u>T</u> GAGGGAAG	4851
		CTTCCCTCAAGCCATCG	4852
	Male-sterile	AAAGGAAAGCTGGAAGAAGAAAACAAGAGCAGTAGTGGTAGTGGT	4853
	DEFA	TCGATGGCTCGAGGGTAGATCCAGATTAAGAGGATAGAGAACCAA	
	Antimhinum majus	ACAAACAGGCAGGTCACCTACTCCAAGAGAA	1
10	Lys5Term AAG-TAG	TTCTCTTGGAGTAGGTGACCTGCCTGTTTGTTTGGTTCTCTATCCT	4854
10	AAG-TAG	CTTAATCTGGATCTACCCTCGAGCCATCGAACCACTACCACTACTG	
		CTCTTGTTTTCTTCCAGCTTTCCTTT CTCGAGGGTAGATCCAG	
			4855
	A A - 1 - 1 - 1	CTGGATCTACCCTCGAG	4856
	Male-sterile DEFA	AAGCTGGAAGAAGAAACAAGAGCAGTAGTGGTAGTGGTTCGATG	4857
		GCTCGAGGGAAGATCTAGATTAAGAGGATAGAGAACCAAACAAA	
	Antimhinum majus Gln7Term	AGGCAGGTCACCTACTCCAAGAGAAAATG	
15	CAG-TAG	CATTICTICTCTTGGAGTAGGTGACCTGCTGTTTGTTTGGTTCTC	4858
	07.0 77.0	TATCCTCTTAATCTAGATCTTCCCTCGAGCCATCGAACCACTACCA CTACTGCTCTTGTTTCTTCTTCCAGCTT	ļ
		GGAAGATCTAGATTAAG	4050
			4859
		CTTAATCTAGATCTTCC	4860
	Male-sterile	GAAGAAGAAACAAGAGCAGTAGTGGTAGTGGTTCGATGGCTCGA	4861
	DEFA	GGGAAGATCCAGATTTAGAGGATAGAGAACCAAACAAACA	
	Antimhinum majus	GTCACCTACTCCAAGAGAAGAAATGGTTTGT	
20	Lys9Term AAG-TAG	ACAAACCATTCTTCTCTTGGAGTAGGTGACCTGCCTGTTTGTT	4862
20	MG-1AG	GTTCTCTATCCTCTAAATCTGGATCTTCCCTCGAGCCATCGAACCA	
		CTACCACTACTGCTCTTGTTTTCTTCTTC TCCAGATT <u>T</u> AGAGGATA	
			4863
		TATCCTCT <u>A</u> AATCTGGA	4864
	Male-sterile	TCAGTAATTCTTAAGATCTCAAACTTTGAGCAAAAAGAAAAAAAA	4865
	AP3	TATGGCTCGTGGGTAGATCCAGATCAAGAGAATAGAGAACCAAAC	
	Nicotiana tabacum	AAACAGACAAGTCACTTATTCTAAGAGAA	
25	Lys5Term AAG-TAG	TTCTCTTAGAATAAGTGACTTGTCTGTTTGTTTGGTTCTCTATTCTC	4866
	MOING	TTGATCTGGATCTAACAATTACTCA	1
		AAAGTTTGAGATCTTAAGAATTACTGA	
		CTCGTGGG <u>T</u> AGATCCAG	4867
		CTGGATCTACCCACGAG	4868

Male-sterile	ATTCTTAAGATCTCAAACTTTGAGCAAAAAGAAAAAAAAA	4869
AP3	TCGTGGGAAGATC <u>T</u> AGATCAAGAGAATAGAGAACCAAACAAACAGA	
Nicotiana tabacum	CAAGTCACTTATTCTAAGAGAAGAAATG	
GIn7Term	CATTTCTTCTCTTAGAATAAGTGACTTGTCTGTTTGTTTG	4870
CAG-TAG	ATTCTCTTGATCTAGATCTTCCCACGAGCCATAGTTTTTTTT	
•	TTGCTCAAAGTTTGAGATCTTAAGAAT	
	GGAAGATC <u>T</u> AGATCAAG	4871
	CTTGATCT <u>A</u> GATCTTCC	4872
Male-sterile	AAGATCTCAAACTTTGAGCAAAAAGAAAAAAAAAAACTATGGCTCGTG	4873
AP3	GGAAGATCCAGATC <u>T</u> AGAGAATAGAGAACCAAACAAACAGACAAGT	
Nicotiana tabacum	CACTTATTCTAAGAGAAGAAATGGACTTT	
Lys9Term	AAAGTCCATTTCTTCTCTTAGAATAAGTGACTTGTCTGTTTGTT	4874
AAG-TAG	TTCTCTATTCTCTAGATCTGGATCTTCCCACGAGCCATAGTTTTTTT	
	TTCTTTTTGCTCAAAGTTTGAGATCTT	
·	TCCAGATC <u>T</u> AGAGAATA	4875
	TATTCTCT <u>A</u> GATCTGGA	4876
Male-sterile	ATCTCAAACTTTGAGCAAAAAGAAAAAAAAAACTATGGCTCGTGGGA	4877
AP3	AGATCCAGATCAAGTGAATAGAGAACCAAACAAACAGACAAGTCAC	
Nicotiana tabacum	TTATTCTAAGAGAAGAAATGGACTTTTCA	
Arg10Term	TGAAAAGTCCATTTCTTCTCTTAGAATAAGTGACTTGTCTGTTTGTT	4878
AGA-TGA	TGGTTCTCTATTCACTTGATCTGGATCTTCCCACGAGCCATAGTTT	
	TTTTTCTTTTTGCTCAAAGTTTGAGAT	
	AGATCAAG <u>T</u> GAATAGAG	4879
	CTCTATTCACTTGATCT	4880
Male-sterile	GGCTCGAGGAAAGATCCAGATCAAGAGAATAGAGAACACAACGAA	4881
AP3	CAGACAAGTAACTTA <u>G</u> TCAAAACGAAGGGATGGTCTTTTCAAGAAG	
Medicago sativa	GCCAATGAGCTCACTGTTCTTTGTGATGCT	
Tyr21Term	AGCATCACAAAGAACAGTGAGCTCATTGGCCTTCTTGAAAAGACCA	4882
TAC-TAG	TCCCTTCGTTTTGACTAAGTTACTTGTCTGTTCGTTGTGTTCTCTAT	
	TCTCTTGATCTGGATCTTTCCTCGAGCC	
	GTAACTTA <u>G</u> TCAAAACG	4883
	CGTTTTGACTAAGTTAC	4884
Male-sterile	CTCGAGGAAAGATCCAGATCAAGAGAATAGAGAACACAACGAACA	4885
AP3	GACAAGTAACTTACT <u>G</u> AAAACGAAGGGATGGTCTTTTCAAGAAGGC	
Medicago sativa	CAATGAGCTCACTGTTCTTTGTGATGCTAA	
Ser22Term TCA-TGA	TTAGCATCACAAAGAACAGTGAGCTCATTGGCCTTCTTGAAAAGAC	4886
	CATCCCTTCGTTTTCAGTAAGTTACTTGTCTGTTCGTTGTGTTCTCT	
	ATTCTCTTGATCTGGATCTTTCCTCGAG	
	AACTTACT <u>G</u> AAAACGAA	4887
	TTCGTTTTCAGTAAGTT	4888

Male-sterile AP3 Medicago sativa	CGAGGAAAGATCCAGATCAAGAGAATAGAGAACACAACGAACAGA CAAGTAACTTACTCA <u>T</u> AACGAAGGGATGGTCTTTTCAAGAAGGCCA ATGAGCTCACTGTTCTTTGTGATGCTAAGG	
Lys23Term AAA-TAA	CCTTAGCATCACAAAGAACAGTGAGCTCATTGGCCTTCTTGAAAAG ACCATCCCTTCGTTATGAGTAAGTTACTTGTCTGTTCGTTGTTCT CTATTCTCTTGATCTGGATCTTTCCTCG	4890
	CTTACTCATAACGAAGG	4891
	CCTTCGTTATGAGTAAG	4892
Male-sterile AP3 Medicago sativa	GGAAAGATCCAGATCAAGAGAATAGAGAACACAACGAACAA GTAACTTACTCAAAA <u>T</u> GAAGGGATGGTCTTTTCAAGAAGGCCAATG AGCTCACTGTTCTTTGTGATGCTAAGGTTT	4893
Arg24Term CGA-TGA	AAACCTTAGCATCACAAAGAACAGTGAGCTCATTGGCCTTCTTGAA AAGACCATCCCTTCATTTTGAGTAAGTTACTTGTCTGTTCGTTGTGT TCTCTATTCTCTTGATCTGGATCTTTCC	4894
	ACTCAAAA <u>T</u> GAAGGGAT	4895
	ATCCCTTC <u>A</u> TTTTGAGT	4896
Male-sterile DEF4 Solanum tuberosum	GGCTCGTGGTAAGATCCAGATCAAGAAAATAGAAAACCAAACAAA	4897
Tyr21Term TAT-TAG	AGCATCACAAAGAACTGTAAGTTCATTAGCCTTCTTGAATAGCCCA TITCTTCTCTTTGACTAAGTCACTTGCCTATTTGTTTGGTTTCTATT TTCTTGATCTGGATCTTACCACGAGCC	4898
·	GTGACTTA <u>G</u> TCAAAGAG	4899
	CTCTTTGA <u>C</u> TAAGTCAC	4900
Male-sterile DEF4 Solanum tuberosum	CTCGTGGTAAGATCCAGATCAAGAAAATAGAAAACCAAACAAA	4901
Ser22Term TCA-TGA	TTAGCATCACAAAGAACTGTAAGTTCATTAGCCTTCTTGAATAGCC CATTTCTTCTCTTC	4902
	GACTTATT <u>G</u> AAAGAGAA	4903
	TTCTCTTT <u>C</u> AATAAGTC	4904
Male-sterile DEF4 Solanum tuberosum	CGTGGTAAGATCCAGATCAAGAAAATAGAAAACCAAACAAA	4905
Lys23Term AAG-TAG	CTITAGCATCACAAAGAACTGTAAGTTCATTAGCCTTCTTGAATAGC CCATTTCTTCTCTATGAATAAGTCACTTGCCTATTTGTTTG	4906
	CTTATTCATAGAGAAGA	4907
	TCTTCTCTATGAATAAG	4908

Male-sterile	GGTAAGATCCAGATCAAGAAAATAGAAAACCAAACAAATAGGCAAG	4909
DEF4	TGACTTATTCAAAGTGAAGAAATGGGCTATTCAAGAAGGCTAATGA	
Solanum tuberosum	ACTTACAGTTCTTTGTGATGCTAAAGTTT	
Arg24Term	AAACTTTAGCATCACAAAGAACTGTAAGTTCATTAGCCTTCTTGAAT	4910
AGA-TGA	AGCCCATTTCTTCACTTTGAATAAGTCACTTGCCTATTTGTTTG	
	TTCTATTTTCTTGATCTGGATCTTACC	
	ATTCAAAG <u>T</u> GAAGAAAT	4911
	ATTTCTTC <u>A</u> CTTTGAAT	4912
Male-sterile	GCTAATGAACTTACTGTTCTTTGTGATGCTAAAGTTTCAATTGTTAT	4913
AP3	GATTTCTAGTACTTGAAAACTTCATGAGTTTATAAGTCCCTCTATCA	
Lycopersicon	CGACCAAACAATTGTTCGATCTGTACC	
esculentum	GGTACAGATCGAACAATTGTTTGGTCGTGATAGAGGGACTTATAAA	4914
Giy27Term	CTCATGAAGTTTTC <u>A</u> AGTACTAGAAATCATAACAATTGAAACTTTAG	
GGA-TGA	CATCACAAAGAACAGTAAGTTCATTAGC	
	CTAGTACT <u>T</u> GAAAACTT	4915
	AAGTTTTC <u>A</u> AGTACTAG	4916
Male-sterile	AATGAACTTACTGTTCTTTGTGATGCTAAAGTTTCAATTGTTATGAT	4917
AP3	TTCTAGTACTGGATAACTTCATGAGTTTATAAGTCCCTCTATCACGA	
Lycopersicon	CCAAACAATTGTTCGATCTGTACCAGA	
esculentum	TCTGGTACAGATCGAACAATTGTTTGGTCGTGATAGAGGGACTTAT	491
Lys28Term	AAACTCATGAAGTTATCCAGTACTAGAAATCATAACAATTGAAACTT	
AAA-TAA	TAGCATCACAAAGAACAGTAAGTTCATT	
•	GTACTGGA <u>T</u> AACTTCAT	491
	ATGAAGTT <u>A</u> TCCAGTAC	492
Male-sterile	ACTGTTCTTTGTGATGCTAAAGTTTCAATTGTTATGATTTCTAGTAC	492
AP3	TGGAAAACTTCAT <u>T</u> AGTTTATAAGTCCCTCTATCACGACCAAACAAT	
Lycopersicon	TGTTCGATCTGTACCAGAAGACTATTG	
esculentum	CAATAGTCTTCTGGTACAGATCGAACAATTGTTTGGTCGTGATAGA	492
Glu31Term	GGGACTTATAAACT <u>A</u> ATGAAGTTTTCCAGTACTAGAAATCATAACAA	
GAG-TAG	TTGAAACTITAGCATCACAAAGAACAGT	
	AACTTCAT <u>T</u> AGTTTATA	492
	TATAAACT <u>A</u> ATGAAGTT	492
Male-sterile	ATTGTTATGATTTCTAGTACTGGAAAACTTCATGAGTTTATAAGTCC	492
AP3	CTCTATCACGACCTAACAATTGTTCGATCTGTACCAGAAGACTATT	
Lycopersicon	GGAGTTGATATTTGGACTACTCACTATG	455
esculentum	CATAGTGAGTAGTCCAAATATCAACTCCAATAGTCTTCTGGTACAG	492
Lys40Term	ATCGAACAATTGTT <u>A</u> GGTCGTGATAGAGGGACTTATAAACTCATGA	
AAA-TAA	AGTTTTCCAGTACTAGAAATCATAACAAT	
	TCACGACC <u>T</u> AACAATTG	492
	CAATTGTT <u>A</u> GGTCGTGA	492

Male-sterile AP3	GGGGCGGGAAGATTGAGATAAAGCGGATCGAGAACGCCACCA	4929
Triticum aestivum	ACAGGCAGGTGACCTAGTCCAAGCGCCGGTCGGGGATCATGAAG AAGGCGCGGGAGCTCACCGTGCTCTGCGACGCC	
Tyr21Term	GGCGTCGCAGAGCACGGTGAGCTCCCGCGCCTTCTTCATGATCC	4930
TAC-TAG	CCGACCGCCTTGGACTAGGTCACCTGCCTGTTGGTGGCGTTC	
	TCGATCCGCTTTATCTCAATCTTCCCCCGCCCC	
	GTGACCTAGTCCAAGCG	4931
	CGCTTGGA <u>C</u> TAGGTCAC	4932
Male-sterile	CGGGGGAAGATTGAGATAAAGCGGATCGAGAACGCCACCAACAG	4933
AP3	GCAGGTGACCTACTCC <u>T</u> AGCGCCGGTCGGGGATCATGAAGAAGG	
Triticum aestivum	CGCGGGAGCTCACCGTGCTCTGCGACGCCCAGG	
Lys23Term	CCTGGGCGTCGCAGAGCACGGTGAGCTCCCGCGCCTTCTTCATG	4934
AAG-TAG	ATCCCCGACCGGCGCTAGGAGTAGGTCACCTGCCTGTTGGTGGC	1
	GTTCTCGATCCGCTTTATCTCAATCTTCCCCCG	
	CCTACTCC <u>T</u> AGCGCCGG	4935
	CCGCCCTAGGAGTAGG	4936
Male-sterile	TTGAGATAAAGCGGATCGAGAACGCCACCAACAGGCAGGTGACCT	4937
AP3	ACTCCAAGCGCCGGTAGGGGATCATGAAGAAGGCGCGGGAGCTC	
Triticum aestivum	ACCGTGCTCTGCGACGCCCAGGTCGCCATCAT	
Ser26Term	ATGATGGCGACCTGGGCGTCGCAGAGCACGGTGAGCTCCCGCGC	4938
TCG-TAG	CTTCTTCATGATCCCCTACCGGCGCTTGGAGTAGGTCACCTGCCT	
	GTTGGTGGCGTTCTCGATCCGCTTTATCTCAA	
•	GCGCCGGT <u>A</u> GGGGATCA	4939
	TGATCCCCTACCGGCGC	4940
Male-sterile	CGGATCGAGAACGCCACCAACAGGCAGGTGACCTACTCCAAGCG	4941
AP3	CCGGTCGGGGATCATGTAGAAGGCGCGGGAGCTCACCGTGCTCT	
Triticum aestivum	GCGACGCCAGGTCGCCATCATCATGTTCTCCT	i
Lys30Term	AGGAGAACATGATGATGGCGACCTGGGCGTCGCAGAGCACGGTG	4942
AAG-TAG	AGCTCCCGCGCCTTCTACATGATCCCCGACCGGCGCTTGGAGTA	
	GGTCACCTGCTGGTGGCGTTCTCGATCCG	
	GGATCATG <u>T</u> AGAAGGCG	4943
	CGCCTTCT <u>A</u> CATGATCC	4944
Male-sterile Silky1	GGGGCGCGCAAGATCGAGATCAAGCGGATCGAGAACGCCACCA	4945
Siiky i Zea mays	ACCGCCAGGTGACCTAGTCCAAGCGCCGGACGGGGATCATGAAG	ď
Tyr21Term	AAGGCACGCGAGCTCACCGTGCTCTGCGACGCC	
TAC-TAG	GGCGTCGCAGAGCACGGTGAGCTCGCGTGCCTTCTTCATGATCC	4946
1710-1710	CCGTCCGCCTTCGACCTAGGTCACCTGGCGGTTGGTGGCGTTC	
	TCGATCCGCTTGATCTCGATCTTGCCGCGCCCC	
	GTGACCTAGTCCAAGCG	4947
	CGCTTGGACTAGGTCAC	4948

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		40.10
Male-sterile	CGCGGCAAGATCGAGATCAAGCGGATCGAGAACGCCACCAACCG	4949
Silky1	CCAGGTGACCTACTCCTAGCGCCGGACGGGGATCATGAAGAAGG	
Zea mays	CACGCGAGCTCACCGTGCTCTGCGACGCCCAGG	
Lys23Tem	CCTGGGCGTCGCAGAGCACGGTGAGCTCGCGTGCCTTCTTCATG	4950
AAG-TAG	ATCCCGTCCGGCGCTAGGAGTAGGTCACCTGGCGGTTGGTGGC	
	GTTCTCGATCCGCTTGATCTCGATCTTGCCGCG	
	CCTACTCC <u>T</u> AGCGCCGG	4951
	CCGGCGCTAGGAGTAGG	4952
Male-sterile	CGGATCGAGAACGCCACCAACCGCCAGGTGACCTACTCCAAGCG	4953
Silky1	CCGGACGGGATCATG <u>T</u> AGAAGGCACGCGAGCTCACCGTGCTCT	
Zea mays	GCGACGCCCAGGTCGCCATCATCATGTTCTCCT	
Lys30Term	AGGAGAACATGATGATGGCGACCTGGGCGTCGCAGAGCACGGTG	4954
AAG-TAG	AGCTCGCGTGCCTTCTACATGATCCCCGTCCGGCGCTTGGAGTAG	
	GTCACCTGGCGGTTGGTGGCGTTCTCGATCCG	
	GGATCATG <u>T</u> AGAAGGCA	4955
	TGCCTTCT <u>A</u> CATGATCC	4956
Male-sterile	ATCGAGAACGCCACCAACCGCCAGGTGACCTACTCCAAGCGCCG	4957
Silky1	GACGGGGATCATGAAGTAGGCACGCGAGCTCACCGTGCTCTGCG	
Zea mays	ACGCCCAGGTCGCCATCATCATGTTCTCCTCCA	
Lys31Term	TGGAGGAGAACATGATGATGGCGACCTGGGCGTCGCAGAGCACG	4958
AAG-TAG	GTGAGCTCGCGTGCCTACTTCATGATCCCCGTCCGGCGCTTGGA	
	GTAGGTCACCTGGCGGTTGGTGGCGTTCTCGAT	
	TCATGAAG <u>T</u> AGGCACGC	4959
	GCGTGCCT <u>A</u> CTTCATGA	4960
Male-sterile	IGCTAGCTGCATTGTCCGGCGAGAGAGATAGCTGCTGCAGGGGGC	4961
AP3	GGCCATGGGGAGGGGCTAGATCGAGATCAAGCGGATCGAGAACG	
Oryza sativa	CGACCAACAGGCAGGTGACCTACTCGAAGCGCC	
Lys5Term	GGCGCTTCGAGTAGGTCACCTGCCTGTTGGTCGCGTTCTCGATCC	4962
AAG-TAG	GCTTGATCTCGATCTAGCCCCTCCCCATGGCCGCCCCCTGCAGC	
	AGCTATCTCTCGCCGGACAATGCAGCTAGC	
	GGAGGGCTAGATCGAG	4963
	CTCGATCT <u>A</u> GCCCCTCC	4964
Male-sterile	TGCATTGTCCGGCGAGAGAGATAGCTGCTGCAGGGGGGCGCCAT	4965
AP3	GGGGAGGGCAAGATC <u>T</u> AGATCAAGCGGATCGAGAACGCGACCA	
Oryza sativa	ACAGGCAGGTGACCTACTCGAAGCGCCGCACGG	
Glu7Term	CCGTGCGCGCTTCGAGTAGGTCACCTGCCTGTTGGTCGCGTTCT	4966
GAG-TAG	CGATCCGCTTGATCTAGATCTTGCCCCTCCCCATGGCCGCCCCCT	
	GCAGCAGCTATCTCTCTCGCCGGACAATGCA	
}	GCAAGATC <u>T</u> AGATCAAG	4967
	CTTGATCTAGATCTTGC	4968

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Male-sterile AP3 Oryza sativa	GTCCGCGAGAGAGATAGCTGCTGCAGGGGGCGGCCATGGGGA GGGGCAAGATCGAGATCTAGCGGATCGAGAACGCGACCAACAGG CAGGTGACCTACTCGAAGCGCCGCACGGGGATCA	4969
Lys9Term AAG-TAG	TGATCCCCGTGCGCGCTTCGAGTAGGTCACCTGCCTGTTGGTC GCGTTCTCGATCCGCTAGATCTCGATCTTGCCCCTCCCATGGCC GCCCCCTGCAGCAGCTATCTCTCTCGCCGGAC	4970
<u></u>	TCGAGATC <u>T</u> AGCGGATC	4971
	GATCCGCT <u>A</u> GATCTCGA	4972
Male-sterile AP3 Oryza sativa	GAGAGATAGCTGCTGCAGGGGGCGGCCATGGGGAGGGGCAAGA TCGAGATCAAGCGGATCTAGAACGCGACCAACAGGCAGGTGACCT ACTCGAAGCGCCGCACGGGGATCATGAAGAAGG	4973
Glu12Term GAG-TAG	CCTTCTTCATGATCCCCGTGCGCGCGCTTCGAGTAGGTCACCTGCC TGTTGGTCGCGTTCTAGATCCGCTTGATCTTGCCCCTCC CCATGGCCGCCCCCTGCAGCAGCTATCTCTC	4974
	AGCGGATC <u>T</u> AGAACGCG	4975
	CGCGTTCT <u>A</u> GATCCGCT	4976

Table 15
Oligonucleotides to produce male-sterile plants

Phenotype, Gene, Plant & Targeted Atteration	Altering Oligos	SEQID NO:
Male-sterile AG Arabidopsis thaliana Tyr35Term TAC-TAG	TCTGTACTAATCAAATTTTGCCCTAAACGTTTTTGGCTTTGGAGCA GCAATCACGGCGTAGCAATCGGAGCTAGGAGGAGATTCCTCTCC CTTGAGGAAATCTGGGAGAGGAAAGATCGAA	4977
	TTCGATCTTTCCTCTCCCAGATTTCCTCAAGGGAGGAGGAATCTCCT CCTAGCTCCGATTGCTACGCCGTGATTGCTGCTCCAAAGCCAAAA ACGTTTAGGGCAAAATTTGATTAGTACAGA	4978
	ACGGCGTAGCAATCGGA TCCGATTGCTACGCCGT	4979 4980
Male-sterile AG Arabidopsis thaliana	CTGTACTAATCAAATTTTGCCCTAAACGTTTTTGGCTTTGGAGCAG CAATCACGGCGTACTAATCGGAGCTAGGAGGAGATTCCTCTCCCT TGAGGAAATCTGGGAGAGAGATCGAAA	4981
Gln36Term CAA-TAA	TTTCGATCTTTCCTCCCAGATTTCCTCAAGGGAGAGGAATCTCC TCCTAGCTCCGATTAGTACGCCGTGATTGCTGCTCCAAAGCCAAA AACGTTTAGGGCAAAATTTGATTAGTACAG	4982
	CGGCGTAC <u>T</u> AATCGGAG	4983
	CTCCGATT <u>A</u> GTACGCCG	4984

Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID NO:
Male-sterile AG Arabidopsis thaliana	ACTAATCAAATTTTGCCCTAAACGTTTTTGGCTTTGGAGCAGCAAT CACGGCGTACCAATAGGAGCTAGGAGGAGATTCCTCTCCCTTGA GGAAATCTGGGAGAGGAAAGATCGAAATCAA	4985
Ser37Term TCG-TAG	TTGATTTCGATCTTTCCTCCCAGATTTCCTCAAGGGAGAGGAAT CTCCTCCTAGCTCCTATTGGTACGCCGTGATTGCTGCTCCAAAGC CAAAAACGTTTAGGGCAAAATTTGATTAGT	4986
	GTACCAATAGGAGCTAG CTAGCTCCTATTGGTAC	4987 4988
Male-sterile AG Arabidopsis thaliana	TAATCAAATTTTGCCCTAAACGTTTTTGGCTTTGGAGCAGCAATCA CGGCGTACCAATCGTAGCTAGGAGGAGATTCCTCTCCCTTGAGGA AATCTGGGAGAGGAAAGATCGAAATCAAAC	4989
Glu38Term GAG-TAG	GTTTGATTTCGATCTTTCCTCTCCCAGATTTCCTCAAGGGAGAGGA ATCTCCTCCTAGCTACGATTGGTACGCCGTGATTGCTGCTCCAAA GCCAAAAACGTTTAGGGCAAAATTTGATTA	4990
	ACCAATCG <u>T</u> AGCTAGGA	4991
	TCCTAGCTACGATTGGT	4992
Male-sterile AG Brassica napus	CTCTCCCACTTCTTTTCGGTGGTTTATTCATTTGGTGACGATATCA CAGAAGCAATGGATTAAGGTGGGAGTAGTCACGATGCAGAGAGTA GCAAGAAGATAGGTAGAGGGAAGATAGAGA	4993
Glu3Term GAA-TAA	TCTCTATCTTCCCTCTACCTATCTTCTTGCTACTCTCTGCATCGTG ACTACTCCCACCTTAATCCATTGCTTCTGTGATATCGTCACCAAAT GAATAAACCACCGAAAAGAAGTGGGAGAG	4994
	CAATGGAT <u>T</u> AAGGTGGG	4995
	CCCACCTTAATCCATTG	4996
Male-sterile AG <i>Brassica napus</i>	TATTCATTTGGTGACGATATCACAGAAGCAATGGATGAAGGTGGG AGTAGTCACGATGCA <u>T</u> AGAGTAGCAAGAAGATAGGTAGAGGGAAG ATAGAGATAAAGAGGATAGAGAACACAA	4997
Glu11Term GAG-TAG	TTGTTGTGTTCTCTATCCTCTTTATCTCTATCTTCCCTCTACCTATC TTCTTGCTACTCTATGCATCGTGACTACTCCCACCTTCATCCATTG CTTCTGTGATATCGTCACCAAATGAATA	4998
	ACGATGCA <u>T</u> AGAGTAGC	4999
	GCTACTCT <u>A</u> TGCATCGT	5000
Male-sterile AG Brassica napus	GGTGACGATATCACAGAAGCAATGGATGAAGGTGGGAGTAGTCA CGATGCAGAGAGTAGCTAGAAGATAGGTAGAGGAAGATAGAGAT AAAGAGGATAGAGAACAACAAATCGTCAAG	5001
Lys14Term AAG-TAG	CTTGACGATTTGTTGTGTTCTCTATCCTCTTTATCTCTATCTTCCCT CTACCTATCTTCTAGCTACTCTCTGCATCGTGACTACTCCCACCTT CATCCATTGCTTCTGTGATATCGTCACC	5002
	AGAGTAGC <u>T</u> AGAAGATA	5003
	TATCTTCTAGCTACTCT	5004

Phenotype, Gene Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Male-sterile AG Brassica napus Lys15Term	GACGATATCACAGAAGCAATGGATGAAGGTGGGAGTAGTCACGAT GCAGAGAGTAGCAAGTAGATAGGTAGAGGAAGATAGAGATAAAG AGGATAGAGAACACAAATCGTCAAGTAA	
AAG-TAG	TTACTTGACGATTTGTTGTTGTTCTCTATCCTCTTTATCTCTATCTTC CCTCTACCTATCTACTTGCTACTCTCTGCATCGTGACTACTCCCAC CTTCATCCATTGCTTCTGTGATATCGTC GTAGCAAGTAGATAGGT	
	ACCTATCTACTTGCTAC	5007
Male-sterile AG Lycopersicon	CAACCAAAAACTTAAAAATCTTCTCTTTCCTTTCCTTACAAGGTGA AGTAATGGACTTC <u>T</u> AAAGTGATCTAACCAGAGAGATCTCACCACAA AGGAAACTAGGAAGGGGGAAAATTGAGA	5008
esculentum Glu4Term CAA-TAA	TCTCAATTTTCCCCCTTCCTAGTTTCCTTTGTGGTGAGATCTCTCT GGTTAGATCACTTTAGAAGTCCATTACTTCACCTTGTAAGGAAAGG AAAGAGAAGATTTTTAAGTTTTTTGGTTG	5010
	TGGACTTCTAAAGTGAT	5011
Malastail	ATCACTTT <u>A</u> GAAGTCCA	5012
Male-sterile AG Lycopersicon esculentum	AAAATCTTCTCTTTCCTTTCCTTACAAGGTGAAGTAATGGACTTCC AAAGTGATCTAACCTGAGAGATCTCACCACAAAGGAAACTAGGAA GGGGGAAAATTGAGATCAAAAGGATCGAAA	5013
Arg9Term AGA-TGA	TITCGATCCTTTTGATCTCAATTTTCCCCCTTCCTAGTTTCCTTTGT GGTGAGATCTCTCAGGTTAGATCACTTTGGAAGTCCATTACTTCAC CTTGTAAGGAAAGGA	5014
	ATCTAACCTGAGAGATC	5015
	GATCTCTCAGGTTAGAT	5016
Male-sterile AG Lycopersicon	ATCTTCTCTTTCCTTTCCTTACAAGGTGAAGTAATGGACTTCCAAA GTGATCTAACCAGATAGATCTCACCACAAAGGAAACTAGGAAGGG GGAAAATTGAGATCAAAAGGATCGAAAACA	5017
esculentum Glu10Term GAG-TAG	TGTTTTCGATCCTTTTGATCTCAATTTTCCCCCTTCCTAGTTTCCTT TGTGGTGAGATCTATCTGGTTAGATCACTTTGGAAGTCCATTACTT CACCTTGTAAGGAAAGGA	5018
	TAACCAGA <u>T</u> AGATCTCA	5019
	TGAGATCT <u>A</u> TCTGGTTA	5020
lale-sterile G /copersicon	CTTTCCTTCCTTACAAGGTGAAGTAATGGACTTCCAAAGTGATCT AACCAGAGAGATCTGACCACAAAGGAAACTAGGAAGGGGGAAAAT TGAGATCAAAAGGATCGAAAACACGACGAA	5021
sculentum er12Term CA-TGA	TTCGTCGTGTTTTCGATCCTTTTGATCTCAATTTTCCCCCTTCCTA GTTTCCTTTGTGGTCAGATCTCTCTGGTTAGATCACTTTGGAAGTC CATTACTTCACCTTGTAAGGAAAGGA	5022
	AGAGATCT <u>G</u> ACCACAAA	5023

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	TTTGTGGT <u>C</u> AGATCTCT	5024
Male-sterile NAG1 Nicotiana tabacum	GTACTCTCTATTTTCATCTTCCAACCCTTTCTTTCCTTACCAGGTGA AAGTATGGACTTCTAAAGTGATCTAACAAGAGAGATCTCTCCACAA AGGAAACTGGGAAGAGGAAAGATTGAGA	5025
GIn4Term CAA-TAA	TCTCAATCTTTCCTCTTCCCAGTTTCCTTTGTGGAGAGATCTCTCT TGTTAGATCACTTTAGAAGTCCATACTTTCACCTGGTAAGGAAAGA AAGGGTTGGAAGATGAAAATAGAGAGTAC	5026
	TGGACTTC <u>T</u> AAAGTGAT	5027
	ATCACTTTAGAAGTCCA	5028
Male-sterile NAG1 Nicotiana tabacum	ATCTTCCAACCCTTTCTTTCCTTACCAGGTGAAAGTATGGACTTCC AAAGTGATCTAACATGAGAGATCTCTCCACAAAGGAAACTGGGAA GAGGAAAGATTGAGATCAAACGGATCGAAA	5029
Arg9Term AGA-TGA	TTTCGATCCGTTTGATCTCAATCTTTCCTCTTCCCAGTTTCCTTTGT GGAGAGATCTCTCATGTTAGATCACTTTGGAAGTCCATACTTTCAC CTGGTAAGGAAAGAAAGGGTTGGAAGAT	5030
	ATCTAACA <u>T</u> GAGAGATC	5031
	GATCTCTCATGTTAGAT	5032
Male-sterile NAG1 Nicotiana tabacum	TTCCAACCCTTTCTTTCCTTACCAGGTGAAAGTATGGACTTCCAAA GTGATCTAACAAGATAGATCTCTCCACAAAGGAAACTGGGAAGAG GAAAGATTGAGATCAAACGGATCGAAAACA	5033
Glu10Term GAG-TAG	TGTTTTCGATCCGTTTGATCTCAATCTTTCCTCTTCCCAGTTTCCTT TGTGGAGAGATCTATCTTGTTAGATCACTTTGGAAGTCCATACTTT CACCTGGTAAGGAAAGAAAGGGTTGGAA	5034
	TAACAAGA <u>T</u> AGATCTCT	5035
	AGAGATCT <u>A</u> TCTTGTTA	5036
Male-sterile NAG1 Nicotiana tabacum	CTTTCCTTACCAGGTGAAAGTATGGACTTCCAAAGTGATCTAACAA GAGAGATCTCTCCATAAAGGAAACTGGGAAGAGGAAAGATTGAGA TCAAACGGATCGAAAACACAACGAATCGTC	5037
Gin14Term CAA-TAA	GACGATTCGTTGTGTTTTCGATCCGTTTGATCTCAATCTTTCCTCT TCCCAGTTTCCTTTATGGAGAGATCTCTCTTGTTAGATCACTTTGG AAGTCCATACTTTCACCTGGTAAGGAAAG	5038
	TCTCTCCATAAAGGAAA	5039
	TTTCCTTT <u>A</u> TGGAGAGA	5040
Male-sterile AG Rosa hybrida	GCCTATGAAAACAAACCCAACACGGTCCTGGACGCTGATGCCCAA AGAAGATTGGGAAGGTGAAAGATCGAGATCAAGCGGATCGAAAAC ACCACCAATCGTCAAGTCACCTTCTGCAAAA	5041
Gly22Term GGA-TGA	TTTTGCAGAAGGTGACTTGACGATTGGTGGTGTTTTCGATCCGCT TGATCTCGATCTTTCACCTTCCCAATCTTCTTTGGGCATCAGCGTC CAGGACCGTGTTGGGTTTGTTTTCATAGGC	5042
	TGGGAAGGTGAAAGATC	5043
•	GATCTITCACCTTCCCA	5044

Phenotype, Gene, Plant & Targeted Alteration	Aftering Oligos	SEQ ID NO:
Male-sterile AG Rosa hybrida	TATGAAAACAAACCCAACACGGTCCTGGACGCTGATGCCCAAAGA AGATTGGGAAGGGGATAGATCGAGATCAAGCGGATCGAAAACAC CACCAATCGTCAAGTCACCTTCTGCAAAAGGC	5045
Lys23Term AAG-TAG	GCCTTTTGCAGAAGGTGACTTGACGATTGGTGGTGTTTTCGATCC GCTTGATCTCGATCTATCCCCTTCCCAATCTTCTTTGGGCATCAGC GTCCAGGACCGTGTTGGGTTTGTTTTCATA	5046
	GAAGGGGATAGATCGAG	5047
Male-sterile	CTCGATCTATCCCCTTC	5048
AG Rosa hybrida	AACAAACCCAACACGGTCCTGGACGCTGATGCCCAAAGAAGATTG GGAAGGGGAAAGATCTAGATCAAGCGGATCGAAAACACCACCAAT CGTCAAGTCACCTTCTGCAAAAGGCGCAATG	5049
Glu25Term GAG-TAG	CATTGCGCCTTTTGCAGAAGGTGACTTGACGATTGGTGGTGTTTT CGATCCGCTTGATCTAGATCTTTCCCCTTCCCAATCTTCTTTGGGC ATCAGCGTCCAGGACCGTGTTGGGTTTGTT	5050
	GAAAGATC <u>T</u> AGATCAAG	5051
	CTTGATCT <u>A</u> GATCTTTC	5052
Male-sterile AG Rosa hybrida	CCCAACACGGTCCTGGACGCTGATGCCCAAAGAAGATTGGGAAG GGGAAAGATCGAGATCTAGCGGATCGAAAACACCACCAATCGTCA AGTCACCTTCTGCAAAAGGCGCAATGGTTTGC	5053
Lys27 AAG-TAG	GCAAACCATTGCGCCTTTTGCAGAAGGTGACTTGACGATTGGTGG TGTTTTCGATCCGCTAGATCTCGATCTTTCCCCTTCCCAATCTTCT TTGGGCATCAGCGTCCAGGACCGTGTTGGG	5054
	TCGAGATC <u>T</u> AGCGGATC	5055
	GATCCGCTAGATCTCGA	5056
Male-sterile far Antirrhinum majus	CAATTGCCTGTTTTTATTTTTTTTTTTTTTTTTGACTAAGTAGAAATGGC GTCTCTAAGCGATTAATCGACCGAGGTATCGCCCGAGAGGAAAAT CGGGAGAGGAAAGATCGAGATCAAACGGA	5057
GIn7Term CAA-TAA	TCCGTTTGATCTCGATCTTTCCTCTCCCGATTTTCCTCTCGGGCGA TACCTCGGTCGATTAATCGCTTAGAGACGCCATTTCTACTTAGTCA AAAAGAAAAAAAAAA	5058
	TAAGCGATTAATCGACC	5059
	GGTCGATT <u>A</u> ATCGCTTA	5060
Male-sterile far Antirrhinum majus	GTTTTATTTTTTCTTTTGACTAAGTAGAAATGGCGTCTCTAAG CGATCAATCGACC <u>T</u> AGGTATCGCCCGAGAGGAAAATCGGGAGAG GAAAGATCGAGATCAAACGGATCGAAAACA	5061
Glu10Term GAG-TAG	TGTTTCGATCCGTTTGATCTCGATCTTTCCTCCCGATTTTCCTCTCGGGCGATACCTAGGGCGATTGATCGCTTAGAGACGCCATTTCTACTTAGTCAAAAAGAAAAAAAA	5062
·	AATCGACC <u>T</u> AGGTATCG	5063
	CGATACCTAGGTCGATT	5064

Phenotype. Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Male-sterile far Antirrhinum majus	TTTCTTTTTGACTAAGTAGAAATGGCGTCTCTAAGCGATCAATCGA CCGAGGTATCGCCCTAGAGGAAAATCGGGAGAGGAAAGATCGAG ATCAAACGGATCGAAAACAAAAC	5065
Glu14Term GAG-TAG	GTTGATTTGTTTTCGATCCGTTTGATCTCGATCTTTCCTCTC CCGATTTTCCTCTAGGGCGATACCTCGGTCGATTGATCGCTTAGA GACGCCATTTCTACTTAGTCAAAAAGAAA	5066
·	TATCGCCCTAGAGGAAA TTTCCTCTAGGGCGATA	5067 5068
Male-sterile	TTTGACTAAGTAGAAATGGCGTCTCTAAGCGATCAATCGACCGAG	5069
far Antirrhinum majus	GTATCGCCCGAGAGGTAAATCGGGAGAGAGATCAATCGACCGAG ACGGATCGAAAACAAAAC	5009
Lys16Term AAA-TAA	TAACCTGTTGATTTGTTTTGTTTTCGATCCGTTTGATCTCGATCTTT CCTCTCCCGATTTACCTCCGGGCGATACCTCGGTCGATTGATCG CTTAGAGACGCCATTTCTACTTAGTCAAA	5070
	CCGAGAGGTAAATCGGG	5071
	CCCGATTTACCTCTCGG	5072
Male-sterile AG Cucumis sativus	TGTCCAAGCATTATCAGTCACCACTCACAAGAATGATTAAGGAAGA AGGAAAGGGTAAGTAGCAAATAAAGGGGATGTTCCAGAATCAAGA AGAGAAGATGTCAGACTCGCCTCAGAGGAA	5073
Leu21Term TTG-TAG	TTCCTCTGAGGCGAGTCTGACATCTTCTCTTCTTGATTCTGGAACA TCCCCTTTATTTGCTACTTACCCTTTCCTTCCTTAATCATTCTT GTGAGTGGTGACTGATAATGCTTGGACA	5074
	GGGTAAGT <u>A</u> GCAAATAA	5075
	TTATTTGC <u>T</u> ACTTACCC	5076
Male-sterile AG Cucumis sativus	TCCAAGCATTATCAGTCACCACTCACAAGAATGATTAAGGAAGAAG GAAAGGGTAAGTTG <u>T</u> AAATAAAGGGGATGTTCCAGAATCAAGAAG AGAAGATGTCAGACTCGCCTCAGAGGAAGA	5077
Gin22Term CAA-TAA	TCTTCCTCTGAGGCGAGTCTGACATCTTCTTCTTCATTCTGGAA CATCCCCTTTATTTACAACTTACCCTTTCCTTCCTTAATCATTC TTGTGAGTGGTGACTGATAATGCTTGGA	5078
	GTAAGTTG <u>T</u> AAATAAAG	5079
	CTTTATTT <u>A</u> CAACTTAC	5080
Male-sterile AG Cucumis sativus	CATTATCAGTCACCACTCACAAGAATGATTAAGGAAGAAGGAAAG GGTAAGTTGCAAATA <u>T</u> AGGGGATGTTCCAGAATCAAGAAGAGAAG ATGTCAGACTCGCCTCAGAGGAAGATGGGAA	5081
Lys24Term AAG-TAG	TTCCCATCTTCCTCTGAGGCGAGTCTGACATCTTCTTCTTGATT CTGGAACATCCCCTATATTTGCAACTTACCCTTTCCTTCC	5082
	TGCAAATA <u>T</u> AGGGGATG	5083
	CATCCCCTATATTTGCA	5084

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ IE
Male-sterile AG Cucumis sativus Gln28Term	CCACTCACAAGAATGATTAAGGAAGAAGGAAAGGGTAAGTTGCAA ATAAAGGGGATGTTCTAGAATCAAGAAGAAGAAGATGTCAGACTCG CCTCAGAGGAAGATGGGAAGAGGAAAGATTG	5085
CAG-TAG	CAATCTTCCTCTCCCATCTTCCTCTGAGGCGAGTCTGACATCTT CTCTTCTTGATTCTAGAACATCCCCTTTATTTGCAACTTACCCTTTC CTTCTTCCTTAATCATTCTTGTGAGTGG	5086
·	GGATGTTC <u>T</u> AGAATCAA TTGATTCT <u>A</u> GAACATCC	5087
Male-sterile		5088
AG  Zea mays  Cys10Term	CCACCACCACCACCACCACCACCACCATGCTCAACATGAT GACTGATCTGAGCTGAG	5089
TGC-TGA	CCTGTCGCCGGAGCCCGTCGGCGCCGCCGCCACCTGCTCCTTG ACCTTGGACGACGGCCCTCAGCTCAG	5090
	GACGGCCTCAGCTCAG	5091
Male-sterile		5092
AG Zea mays Ser13Term	ACCACCACCACCACCACCACCATGCTCAACATGATGACTGATC TGAGCTGCGGGCCGTAGTCCAAGGTCAAGGAGCAGGTGGCGGC GGCGCCGACGGGCTCCGGCGACAGGCAGGGGCA	5093
TCG-TAG	TGCCCCTGCCTGTCGCCGGAGCCCGTCGGCGCCGCCACCT GCTCCTTGACCTTGGACTACGGCCCGCAGCTCAGATCAGTCATCA TGTTGAGCATGGTGGTGGTGGTGGTGGTGGT	5094
	CGGGCCGT <u>A</u> GTCCAAGG	5095
Male-sterile	CCTTGGACTACGGCCCG	5096
Maie-sterile AG Zea mays Lys15Term	CACCACCACCACCACCATGCTCAACATGATGACTGATCTGAGC TGCGGGCCGTCGTCCTAGGTCAAGGAGCAGGTGGCGGCGCGCCCCGACGGGCTCCGGCGACAGGCAGG	5097
AG-TAG	TCCCCTGCCCTGCCTGTCGCCGGAGCCCGTCGGCGCCGCCGCCACCTGCTCCTTGACCTAGGACGACGGCCCGCAGCTCAGATCAGTCATCATGTTGAGCATGGTGTGGTGGTGGTGGTGGTG	5098
	CTTCACCTACCACCACC	5099
/ale-sterile	CTTGACCTAGGACGACG	5100
AG Zea mays ys17Term	CACCACCACACATGCTCAACATGATGACTGATCTGAGCTGCGGG CCGTCGTCCAAGGTC <u>T</u> AGGAGCAGGTGGCGGCGCGCCGACGG GCTCCGGCGACAGGCAGGGGAGAGGCA	5101
AG-TAG	TGCCTCTCCCTGCCCTGCCTGTCGCCGAGCCCGTCGGCGCCCGCC	5102
	CCAAGGTCTAGGAGCAG	5103
	CTGCTCCTAGACCTTGG	5104

Phenotype, Gene, Plant & Targeted Alteration	Attering Oligos	SEQID NO:
Male-sterile AG Zea mays	TCCTACCTTTCTCCTTCAGACCTCAAAATCTGTGTGATAGGAACA AGAGCATGCACATCTGAGAAGAGGAGGCTACACCATCCACAGTAA CAGGCATCATGTCGACCCTGACTTCGGCGG	5105
Arg4Term CGA-TGA	CCGCCGAAGTCAGGGTCGACATGATGCCTGTTACTGTGGATGGT GTAGCCTCCTCTTCTCAGATGTGCATGCTCTTGTTCCTATCACACA GATTTTGAGGTCTGAAGGAGAAAAGGTAGGA	5106
	TGCACATCTGAGAAGAG	5107 5108
Male-sterile AG	TACCTTTCTCAGACCTCAAAATCTGTGTGATAGGAACAAGA GCATGCACATCCGATAAGAGGAGGCTACACCATCCACAGTAACAG	5109
Zea mays Glu5Term GAA-TAA	GCATCATGTCGACCCTGACTTCGGCGGGGC  GCCCCGCCGAAGTCAGGGTCGACATGATGCCTGTTACTGTGGAT GGTGTAGCCTCCTCTTATCGGATGTGCATGCTCTTGTTCCTATCA CACAGATTTTGAGGTCTGAAGGAGAAAAGGTA	5110
	ACATCCGA <u>T</u> AAGAGGAG	5111
	CTCCTCTTATCGGATGT	5112
Male-sterile AG	CTTTTCTCCTTCAGACCTCAAAATCTGTGTGATAGGAACAAGAGCA TGCACATCCGAGAATAGGAGGCTACACCATCCACAGTAACAGGCA TCATGTCGACCCTGACTTCGGCGGGGCAGC	5113
Zea mays Glu6Term GAG-TAG	GCTGCCCGCCGAAGTCAGGGTCGACATGATGCCTGTTACTGTG GATGGTGTAGCCTCCTATTCTCGGATGTGCATGCTCTTGTTCCTA TCACACAGATTTTGAGGTCTGAAGGAGAAAAG	5114
	TCCGAGAA <u>T</u> AGGAGGCT	5115
	AGCCTCCT <u>A</u> TTCTCGGA	5116
Male-sterile AG Zea mays	TTCTCCTTCAGACCTCAAAATCTGTGTGATAGGAACAAGAGCATGC ACATCCGAGAAGAGTAGGCTACACCATCCACAGTAACAGGCATCA TGTCGACCCTGACTTCGGCGGGGCAGCAGA	5117
Glu7Term GAG-TAG	TCTGCTGCCCGCCGAAGTCAGGGTCGACATGATGCCTGTTACT GTGGATGGTGTAGCCTACTCTTCTCGGATGTGCATGCTCTTGTTC CTATCACACAGATTTTGAGGTCTGAAGGAGAA	5118
	GAGAAGAG <u>T</u> AGGCTACA	5119
	TGTAGCCTACTCTTCTC	5120
Male-sterile AG Onyza sativa	GCTGGGTCAGGATCGTCGGCGGCGGGGGGGGGGGGGGGG	5121
Lys5Term AAG-TAG	GGCGCTTGCAGAAGGTCACCTGCCGGTTCGTCGTGTTCTCGATC CGCTTTATCTCGATCTACCCCCTCCCCATCTTCTCGCTGCTCCCC GCCGCCACCGCCGACGATCCTGACCCAGC	5122
	GGAGGGGTAGATCGAG	5123
	CTCGATCTACCCCCTCC	5124

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Male-sterile AG Oryza sativa	TCAGGATCGTCGGCGGCGGGGGGGGGGGGAGAAGA TGGGGAGGGGGAAGATCTAGATAAAGCGGATCGAGAACACGACG AACCGGCAGGTGACCTTCTGCAAGCGCCGCAATG	5125
Glu7Term GAG-TAG	CATTGCGGCGCTTGCAGAAGGTCACCTGCCGGTTCGTCGTTC TCGATCCGCTTTATCTAGATCTTCCCCCCTCCCCATCTTCTCGCTG CTCCCCGCCGCCACCGCCGACGATCCTGA	5126
	GGAAGATC <u>T</u> AGATAAAG	5127
	CTTTATCTAGATCTTCC	5128
Male-sterile AG Oryza sativa	TCGTCGGCGGCGGTGGCGGCGGGGAGCAGCGAGAGATGGGGAGGGGGAGACCGAGGAGACCGAGGGGGAGACACGACG	5129
Lys9Term AAG-TAG	GGAGGCCATTGCGGCGCTTGCAGAAGGTCACCTGCCGGTTCGTC GTGTTCTCGATCCGCTATATCTCGATCTTCCCCCTCCCCATCTTCT CGCTGCTCCCCGCCGCCGCCGCCGACGA	5130
	TCGAGATA <u>T</u> AGCGGATC	5131
	GATCCGCT <u>A</u> TATCTCGA	5132
Male-sterile AG Oryza sativa	GCGGTGGCGGCGGGGGGGGGGGGGGGGGGGGGGGGGGG	5133
Glu12Term GAG-TAG	CCTTCTTCAGGAGGCCATTGCGGCGCTTGCAGAAGGTCACCTGC CGGTTCGTCGTGTTCTAGATCCGCTTTATCTCGATCTTCCCCCTC CCCATCTTCTCGCTGCTCCCCGCCGCCACCGC	5134
	AGCGGATC <u>T</u> AGAACACG	5135
	CGTGTTCTAGATCCGCT	5136

Table 16
Oligonucleotides to produce male-sterile plants

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Male-sterile Pl Cucumis sativus	GGGAAGAGGGAAAATAGAAATAAAAAGAATAGAGAACTCAAGCAAT AGACAAGTTACATAGTCAAAGAGAAGAAATGGTATCATCAAAAAAG CCAAAGAAATTACTGTTCTTTGCGATGCT	5137
Tyr21Term TAT-TAG	AGCATCGCAAAGAACAGTAATTTCTTTGGCTTTTTTGATGATACCAT TTCTTCTCTTTGACTAGTAACTTGTCTATTGCTTGAGTTCTCTATTC TTTTTATTTCTATTTTCCCTCTTCCC	5138

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Adiejados	GTTACATAGTCAAAGAG	5139
	CTCTTTGACTATGTAAC	5140
Male-sterile	GAAGAGGGAAAATAGAAATAAAAAGAATAGAGAACTCAAGCAATAG ACAAGTTACATATTGAAAGAGAAGAAATGGTATCATCAAAAAAAGCC	5141
Cucumis sativus Ser22Term FCA-TGA	AAAGAAATTACTGTTCTTTGCGATGCTCA TGAGCATCGCAAAGAACAGTAATTTCTTTGGCTTTTTTGATGATACC ATTTCTTCTCTTTCAATATGTAACTTGTCTATTGCTTGAGTTCTCTAT TCTTTTTATTTCTATTTTCCCTCTTC	5142
	TACATATT <u>G</u> AAAGAGAA	5143
	TTCTCTTT <u>C</u> AATATGTA	5144
Male-sterile Pl	AGAGGGAAAATAGAAATAAAAAGAATAGAGAACTCAAGCAATAGAC AAGTTACATATTCATAGAGAAGAAATGGTATCATCAAAAAAAGCCAAA GAAATTACTGTTCTTTGCGATGCTCAAG	5145
Cucumis sativus Lys23Term AAG-TAG	CTTGAGCATCGCAAAGAACAGTAATTTCTTTGGCTTTTTTGATGATA CCATTTCTTCTCTATGAATATGTAACTTGTCTATTGCTTGAGTTCTC TATTCTTTTTATTTCTATTTTCCCTCT	5146
	CATATTCA <u>T</u> AGAGAAGA	5147
	TCTTCTCT <u>A</u> TGAATATG	5148
Male-sterile Pl	GGGAAAATAGAAATAAAAAGAATAGAGAACTCAAGCAATAGACAAG TTACATATTCAAAGTGAAGAAATGGTATCATCAAAAAAAGCCAAAGAA ATTACTGTTCTTTGCGATGCTCAAGTTT	5149
Cucumis sativus Arg24Term AGA-TGA	AAACTTGAGCATCGCAAAGAACAGTAATTTCTTTGGCTTTTTTGATG ATACCATTTCTTCACTTTGAATATGTAACTTGTCTATTGCTTGAGTT CTCTATTCTTTTTATTTCTATTTTCCC	5150
	ATTCAAAG <u>T</u> GAAGAAAT	515
	ATTTCTTC <u>A</u> CTTTGAAT	515
Male-sterile Pl Malus domestica	GGGACGTGGGAAGGTTGAGATCAAGAGGATTGAGAACTCAAGTAA CAGGCAGGTGACCTAGTCCAAGAGGAGGAATGGGATTATCAAGAA GGCAAAGGAGATCACTGTTCTATGTGATGCT	515
Tyr21Term TAC-TAG	AGCATCACATAGAACAGTGATCTCCTTTGCCTTCTTGATAATCCCA TTCCTCCTCTTGGACTAGGTCACCTGCCTGTTACTTGAGTTCTCAA TCCTCTTGATCTCAACCTTCCCACGTCCC	515
	GTGACCTAGTCCAAGAG	515
	CTCTTGGACTAGGTCAC	515

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Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQID NO:
Male-sterile PI Malus domestica Lys23Term	CGTGGGAAGGTTGAGATCAAGAGGATTGAGAACTCAAGTAACAGG CAGGTGACCTACTCCTAGAGGAGGAATGGGATTATCAAGAAGGCA AAGGAGATCACTGTTCTATGTGATGCTAAAG	
AAG-TAG	CTITAGCATCACATAGAACAGTGATCTCCTTTGCCTTCTTGATAATC CCATTCCTCCTAGGAGTAGGTCACCTGCCTGTTACTTGAGTTCT CAATCCTCTTGATCTCAACCTTCCCACG	
	CCTACTCC <u>T</u> AGAGGAGG CCTCCTCT <u>A</u> GGAGTAGG	5159
Male-sterile PI Malus domestica	AGGATTGAGAACTCAAGTAACAGGCAGGTGACCTACTCCAAGAGG AGGAATGGGATTATCTAGAAGGCAAAGGAGATCACTGTTCTATGTG ATGCTAAAGTATCTCTTATCATTTATTCTA	1
Lys30Term AAG-TAG	TAGAATAAATGATAAGAGATACTTTAGCATCACATAGAACAGTGATC TCCTTTGCCTTCTAGATAATCCCATTCCTCCTCTTGGAGTAGGTCA CCTGCCTGTTACTTGAGTTCTCAATCCT GGATTATCTAGAAGGCA	
	TGCCTTCTAGAAGGCA	5163
Male-sterile Pl Malus domestica	ATTGAGAACTCAAGTAACAGGCAGGTGACCTACTCCAAGAGGAGG AATGGGATTATCAAGTAGGCAAAGGAGATCACTGTTCTATGTGATG CTAAAGTATCTCTTATCATTTATTCTAGCT	5164 5165
Lys31Term AAG-TAG	AGCTAGAATAAATGATAAGAGATACTTTAGCATCACATAGAACAGT GATCTCCTTTGCCTACTTGATAATCCCATTCCTCCTCTTGGAGTAG GTCACCTGCCTGTTACTTGAGTTCTCAAT	5166
	TTATCAAGTAGGCAAAG	5167
Male-sterile	CTTTGCCT <u>A</u> CTTGATAA	5168
globosa  Antinhinum majus Gly2Term	CATTITIACAATAGTTATCTGCAAACAAAACAAGAGAGAAAAACAA AAACAAAAAAATG <u>T</u> GAAGAGGAAAAATTGAGATCAAAAGAATTGAG AACTCAAGCAACAGGCAGGTTACTTACT	5169
GGA-TGA	AGTAAGTAACCTGCCTGTTGCTTGAGTTCTCAATTCTTTTGATCTCA ATTTTTCCTCTTCACATTTTTTTGTTTTTGTTTTTCTCTCTTGTTTTTG TTTGCAGATAACTATTGTAAAAATG	5170
	AAAAAATG <u>T</u> GAAGAGGA TCCTCTC <u>A</u> CATTTTT	5171
Male-sterile		5172
globosa  Antimhinum majus  Arg3Term	TTTACAATAGTTATCTGCAAACAAAACAAGAGAGAAAAAAAA	5173
AGA-TGA	TTGAGTAAGTAACCTGCCTGTTGCTTGAGTTCTCAATTCTTTTGATC TCAATTTTTCCTCATCCCATTTTTTTGTTTTTGTTTTCTCTCTTGTT TTTGTTTGCAGATAACTATTGTAAAA	5174

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID No:
	AAATGGGA <u>T</u> GAGGAAAA	5175
	TTTTCCTCATCT	5176
Male-sterile globosa Antirrhinum majus	TACAATAGTTATCTGCAAACAAAACAAGAGAGAGAAAAACAAAAAAAA	5177
Gly4Term GGA-TGA	TCTTTGAGTAAGTAACCTGCCTGTTGCTTGAGTTCTCAATTCTTTTG ATCTCAATTTTTCATCTTCCCATTTTTTTGTTTTTGTTTTCTCTCTT GTTTTGTTTT	5178
•	TGGGAAGA <u>T</u> GAAAAATT	5179
	AATTTTCATCTTCCCA	5180
Male-sterile globosa Antirrhinum majus	AATAGTTATCTGCAAACAAAAACAAGAGAGAAAAAAAAAA	5181
Lys5Term AAA-TAA	TTCTCTTTGAGTAAGTAACCTGCCTGTTGCTTGAGTTCTCAATTCTT TTGATCTCAATTTATCCTCTTCCCATTTTTTTGTTTTTCTCT CTTGTTTTTGTTTTG	5182
	GAAGAGGA <u>T</u> AAATTGAG	5183
	CTCAATTT <u>A</u> TCCTCTTC	5184
Male-sterile Pl Zea mays	GCTGAGCTCTTGCTGCCCTTGGATCTGTTTGGGAGTGGAGAACGC AGTATGGGGCGCGGCTAGATCAAGATCAAGAGGATCGAGAACTCT ACCAACCGGCAGGTGACCTTCTCCAAGCGCC	5185
Lys5Tem AAG-TAG	GGCGCTTGGAGAAGGTCACCTGCCGGTTGGTAGAGTTCTCGATCC TCTTGATCTTGATCTAGCCGCGCCCCCATACTGCGTTCTCCACTCC CAAACAGATCCAAGGGCAGCAAGAGCTCAGC	5186
	GGCGCGCTAGATCAAG	5187
	CTTGATCTAGCCGCGCC	5188
Male-sterile Pl Zea mays	CTCTTGCTGCCCTTGGATCTGTTTGGGAGTGGAGAACGCAGTATG GGGCGCGGCAAGATCTAGATCAAGAGGATCGAGAACTCTACCAAC CGGCAGGTGACCTTCTCCAAGCGCCGGGCCG	5189
Lys7Term AAG-TAG	CGCCCGCCCTTGGAGAAGGTCACCTGCCGGTTGGTAGAGTTC TCGATCCTCTTGATCTAGATCTTGCCGCGCCCCCATACTGCGTTCTC CACTCCCAAACAGATCCAAGGGCAGCAAGAG	5190
	GCAAGATC <u>T</u> AGATCAAG	5191
	CTTGATCTAGATCTTGC	5192

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Male-sterile Pl Zea mays	CTCTTGCTGCCCTTGGATCTGTTTGGGAGTGGAGAACGCAGTATG GGGCGCGGCAAGATCTAGATCAAGAGGATCGAGAACTCTACCAAC CGGCAGGTGACCTTCTCCAAGCGCCGGGCCG	5193
Lys9Term AAG-TAG	CGGCCCGGCGCTTGGAGAAGGTCACCTGCCGGTTGGTAGAGTTC TCGATCCTCTTGATCTAGATCTTGCCGCGCCCCCATACTGCGTTCTC CACTCCCAAACAGATCCAAGGGCAGCAAGAG	5194
	GCAAGATC <u>T</u> AGATCAAG	5195
	CTTGATCTAGATCTTGC	5196
Male-sterile PI Zea mays	GATCTGTTTGGGAGTGGAGAACGCAGTATGGGGCGCGGCAAGAT CAAGATCAAGAGGATCTAGAACTCTACCAACCGGCAGGTGACCTT CTCCAAGCGCCGGGCCGG	5197
Glu12Term GAG-TAG	CCTTCTTGACCAGTCCGGCCCGGCGCTTGGAGAAGGTCACCTGC CGGTTGGTAGAGTTCTAGATCCTCTTGATCTTGATCTTGCCGCGC CCCATACTGCGTTCTCCACTCCCAAACAGATC	5198
	AGAGGATC <u>T</u> AGAACTCT	5199
	AGAGTTCT <u>A</u> GATCCTCT	5200
Male-sterile PI Zea mays	GCTGAGCTCTTGCTGCCCTTGAATCTGTTAGGGAGTGGAGAACGG AGTATGGGGCGCGGCTAGATCGAGATCAAGAGGATCGAGAACTCT ACCAACCGGCAGGTGACCTTCTCCAAGCGCC	5201
Lys5Term AAG-TAG	GGCGCTTGGAGAAGGTCACCTGCCGGTTGGTAGAGTTCTCGATCC TCTTGATCTCGATCTAGCCGCCCCCATACTCCGTTCTCCACTCC CTAACAGATTCAAGGGCAGCAAGAGCTCAGC	5202
	GGCGCGC <u>T</u> AGATCGAG	5203
	CTCGATCTAGCCGCGCC	5204
Male-sterile PI Zea mays	CTCTTGCTGCCCTTGAATCTGTTAGGGAGTGGAGAACGGAGTATG GGGCGCGGCAAGATCTAGATCAAGAGGATCGAGAACTCTACCAAC CGGCAGGTGACCTTCTCCAAGCGCCGGGCCG	5205
GIU/Term GAG-TAG	CGGCCCGGCGCTTGGAGAAGGTCACCTGCCGGTTGGTAGAGTTC TCGATCCTCTTGATCTAGATCTTGCCGCGCCCCATACTCCGTTCTC CACTCCCTAACAGATTCAAGGGCAGCAAGAG	5206
	GCAAGATC <u>T</u> AGATCAAG	5207
	CTTGATCT <u>A</u> GATCTTGC	5208
Male-sterile Pl Zea mays	CTGCCCTTGAATCTGTTAGGGAGTGGAGAACGGAGTATGGGGCG CGGCAAGATCGAGATCTAGAGGATCGAGAACTCTACCAACCGGCA GGTGACCTTCTCCAAGCGCCGGGCCGG	5209
Lys9Term AAG-TAG	CCAGTCCGGCCCGGCGCTTGGAGAAGGTCACCTGCCGGTTGGTA GAGTTCTCGATCCTCTAGATCTCGATCTTGCCGCGCCCCATACTC CGTTCTCCACTCCCTAACAGATTCAAGGGCAG	5210

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
•	TCGAGATC <u>T</u> AGAGGATC	5211
	GATCCTCTAGATCTCGA	5212
Male-sterile Pl Zea mays	AATCTGTTAGGGAGTGGAGAACGGAGTATGGGGCGCGCAAGAT CGAGATCAAGAGGATCTAGAACTCTACCAACCGGCAGGTGACCTT CTCCAAGCGCCGGGCCGG	5213
Glu12Term GAG-TAG	CCTTCTTGACCAGTCCGGCCCGGCGCTTGGAGAAGGTCACCTGC CGGTTGGTAGAGTTCTAGATCCTCTTGATCTCGATCTTGCCGCGC CCCATACTCCGTTCTCCACTCCCTAACAGATT	5214
	AGAGGATC <u>T</u> AGAACTCT	5215
	AGAGTTCT <u>A</u> GATCCTCT	5216
Male-sterile Pl Oryza sativa	TTGCTGCTAAGCTAGCTGGAGGAAGGAGGAGGAGGAGGAGGAGGAGGAGGATGGGGCGCGGGTAGATCGAGATCAAGAGGATCGAGAACT CCACCAACCGCCAGGTGACCTTCTCCAAGCGCA	5217
Lys5Term AAG-TAG	TGCGCTTGGAGAAGGTCACCTGGCGGTTGGTGGAGTTCTCGATCC TCTTGATCTCGATCTACCCGCGCCCCATCCCGCCTCCTCCTC CTCCTCCTTCCTCCAGCTAGCTTAGCAGCAA	5218
	GGCGCGG <u>T</u> AGATCGAG	5219
	CTCGATCT <u>A</u> CCCGCGCC	5220
Male-sterile Pl Oryza sativa	CTAAGCTAGCTGGAGGAAGGAGGAGGAGGAGGAGGAGGAGGAGGAGGAGG	5221
Glu7Term GAG-TAG	CGCTCCTGCGCTTGGAGAAGGTCACCTGGCGGTTGGTGGAGTTC TCGATCCTCTTGATCTAGATCTTCCCGCGCCCCATCCCGCCTCCT CCTCCTCCTCCTTCCTCCAGCTAGCTTAG	5222
	GGAAGATC <u>T</u> AGATCAAG	5223
	CTTGATCT <u>A</u> GATCTTCC	5224
Male-sterile PI O <i>ryza sativa</i>	TAGCTGGAGGAGGAGGAGGAGGAGGAGGAGGGGGCGGGATGGGGCGCGGGAAGATCGAGATCTAGAGGATCGAGAACTCCACCAACCGCCAGGGTGACCTTCTCCAAGCGCAGGAGCGGGATCC	5225
Lys9Term AAG-TAG	GGATCCCGCTCCTGCGCTTGGAGAAGGTCACCTGGCGGTTGGTG GAGTTCTCGATCCTCTAGATCTCGATCTTCCCGCGCCCCATCCCG CCTCCTCCTCCTCCTCCTCCTCCAGCTA	5226
	TCGAGATC <u>T</u> AGAGGATC	5227
	GATCCTCTAGATCTCGA	5228

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID No:
Male-sterile PI Oryza sativa Glu12Term GAG-TAG	GAAGGAGGAGGAGGAGGAGGGGGGGGGGGAAGA TCGAGATCAAGAGGATCTAGAACTCCACCAACCGCCAGGTGACCT TCTCCAAGCGCAGGAGCGGGATCCTCAAGAAGG	5229
	CCTTCTTGAGGATCCCGCTCCTGCGCTTGGAGAAGGTCACCTGGCGGTTGGTGGAGATCTTCCCGCGCCCCATCCCGCCCTCCTCCTCCTCCTTC	5230
	AGAGGATC <u>T</u> AGAACTCC	5231
	GGAGTTCT <u>A</u> GATCCTCT	5232

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## Example 7

## Engineering plants for abiotic stress tolerance

Environmental stresses, such as drought, increased soil salinity, soil contamination with heavy metals, and extreme temperature, are major factors limiting plant growth and productivity. The worldwide loss in yield of three major cereal crops, rice, maize, and wheat due to water stress (drought) has been estimated to be over ten billion dollars annually and many currently marginal soils could be brought into cultivation if suitable plant varieties were available.

Physiological and biochemical responses to high levels of ionic or nonionic solutes and decreased water potential have been studied in a variety of plants. It is known, for example, that increasing levels of alcohol dehydrogenase can confer enhances flooding resistance in plants. There are also several possible mechanisms to enhance plant salt tolerance. For example, one mechanism underlying the adaptation or tolerance of plants to osmotic stresses is the accumulation of compatible, low molecular weight osmolytes such as sugar alcohols, special amino acids, and glycinebetaine. Such accumulation can be engineered, for example, by removing feedback inhibition on 1-pyrroline-t-carboxylate synthetase, which results in accumulation of proline. Additionally, recent experiments suggest that altering the expression or activity of specific sodium or potassium transporters can confer enhanced salt tolerance.

Plant tolerance of contamination by heavy metals such as lead and aluminum in soils has also been investigated and one mechanism underlying tolerance is the production of dicarboxylic acids such as oxalate and citrate. In addition, individual genes involved in heavy metal sensitivity have been identified.

The attached tables disclose exemplary oligonucleotide base sequences which can be used to generate site-specific mutations that confer stress tolerance in plants.

Table 17
<u>Genome-Altering Oligos Conferring Stress Tolerance</u>

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ II
Salt Tolerance	CGTCTTTTGTGTGGTAGTTGGATGTGACGGTTGCTCAAATGCTT	5233
P5CS Arabidopsis thaliana	GTGACCGATAGCAGT <u>GC</u> TAGAGATAAGGATTTCAGGAAGCAACTT AGTGAAACTGTCAAAGCGATGCTGAGGATGA	
Phe128Ala	TCATCCTCAGCATCGCTTTGACAGTTTCACTAAGTTGCTTCCTGAA	5234
TTT-GCT	ATCCTTATCTCTAGCACTGCTATCGGTCACAAGCATTTGAGCAACC	5234
	GTCACATCCAACTACCACACAAAAAGACG	
	ATAGCAGT <u>GC</u> TAGAGAT	5235
	ATCTCTA <u>GC</u> ACTGCTAT	5236
Salt Tolerance	GAGACTATGTTTGACCAGCTGGATGTGACGGCTGCTCAGCTGCTG	5237
P5CS 1	GTGAATGACAGTAGT <u>GC</u> CAGAGACAAGGAGTTCAGGAAGCAACTT	
Brassica napus	AATGAGACAGTGAAGTCCATGCTTGATTTGA	
Phe128Ala	TCAAATCAAGCATGGACTTCACTGTCTCATTAAGTTGCTTCCTGAA	5238
TTC-GCC	CTCCTTGTCTCTGGCACTACTGTCATTCACCAGCAGCTGAGCAGC	
•	CGTCACATCCAGCTGGTCAAACATAGTCTC	
	ACAGTAGT <u>GC</u> CAGAGAC	5239
	GTCTCTG <u>GC</u> ACTACTGT	5240
Salt Tolerance	GAGACTATGTTTGACCAGATGGATGTGACGGTGGCTCAAATGCTG	5241
P5CS 2	GTGACTGATAGCAGTGTCAGAGATAAGGATTTCAGGAAGCAACTT	
Brassica napus	AGTGAGACAGTCAAAGCTATGCTGAAAATGA	
Phe129Ala	TCATTTTCAGCATAGCTTTGACTGTCTCACTAAGTTGCTTCCTGAA	5242
TTC-GCC	ATCCTTATCTCTGACACTGCTATCAGTCACCAGCATTTGAGCCACC	
	GTCACATCCATCTGGTCAAACATAGTCTC	
	ATAGCAGTGTCAGAGAT	5243
	ATCTCTGACACTGCTAT	5244
Salt Tolerance	GATATGTTGAACCAACTGGATGTCTCGTCATCTCAACTTCTTG	5245
P5CS	TCACCGACAGTGAT <u>GC</u> TGAGAACCCAAAGTTCCGGGAGCAACTCA	
Oryza sativa	CTGAAACTGTTGAGTCATTATTAGATCTTA	
Phe128Ala	TAAGATCTAATAATGACTCAACAGTTTCAGTGAGTTGCTCCCGGAA	5246
TTT-GCT	CTTTGGGTTCTCAGCATCACTGTCGGTGACAAGAAGTTGAGATGA	
	CGAGACATCCAGTTGGTTAAACAACATATC	
	ACAGTGAT <u>GC</u> TGAGAAC	5247
<u>.                                    </u>	GTTCTCAGC ATCACTGT	5248

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Salt Tolerance P5CS Medicago sativa	GATATTTTGTTTAGTCAGCTGGATGTGACATCTGCTCAGCTTCTTG TTACTGACAATGATGCTAGAGACCAAGATTTTAGAAAGCAACTTTC TGAAACTGTGAGATCACTTCTAGCACTAA	5249
Phe128Ala TTT-GCT	TTAGTGCTAGAAGTGATCTCACAGTTTCAGAAAGTTGCTTTCTAAA ATCTTGGTCTCTA <u>GC</u> ATCATTGTCAGTAACAAGAAGCTGAGCAGAT GTCACATCCAGCTGACTAAACAAAATATC	5250
	ACAATGAT <u>GC</u> TAGAGAC	5251
	GTCTCTA <u>GC</u> ATCATTGT	5252
Salt Tolerance P5CS Actinidia deliciosa	GATACATTGTTTAGTCAGCTGGATGTGACATCAGCTCAGCTACTC GTTACTGATAATGATGCTAGGGATCCAGAATTCAGGAAGCAACTTA CTGAAACTGTAGAATCACTATTGAATTTGA	5253
Phe128Ala TTT-GCT	TCAAATTCAATAGTGATTCTACAGTTTCAGTAAGTTGCTTCCTGAAT TCTGGATCCCTA <u>GC</u> ATCATTATCAGTAACGAGTAGCTGAGCTGAT GTCACATCCAGCTGACTAAACAATGTATC	5254
	ATAATGAT <u>GC</u> TAGGGAT	5255
	ATCCCTAGCATCATTAT	5256
Salt Tolerance P5CS Cichorium intybus	GACACACTCTTCAGTCAACTGGATGTGACATCAGCACAGCTTCTT GTAACAGATAATGACGCCAGAAGTCCAGAATTTAGAAAACAACTTA CTGAAACAGTCGATTCTTTATTATCTTATA	5257
Phe122Ala TTC-GCC	TATAAGATAATAAAGAATCGACTGTTTCAGTAAGTTGTTTTCTAAAT TCTGGACTTCTGGCGCTCATTATCTGTTACAAGAAGCTGTGCTGAT GTCACATCCAGTTGACTGAAGAGTGTGTC	5258
	ATAATGAC <u>GC</u> CAGAAGT	5259
	ACTTCTGGCGTCATTAT	5260
Salt Tolerance P5CS Lycopersicon	GATTCTTTGTTCAGTCAGTTGGATGTGACATCAGCTCAGCTTCTGG TGACTGATAATGACGCTAGAGATCCAGATTTTAGGAGACAACTCAA TGACACAGTAAATTCGTTGCTTTCTCTAA	5261
esculentum Phe128Ala TTT-GCT	TTAGAGAAAGCAACGAATTTACTGTGTCATTGAGTTGTCTCCTAAA ATCTGGATCTCTA <u>GC</u> GTCATTATCAGTCACCAGAAGCTGAGCTGA TGTCACATCCAACTGACTGAACAAAGAATC	5262
	ATAATGAC <u>GC</u> TAGAGAT	5263
	ATCTCTA <u>GC</u> GTCATTAT	5264
Salt Tolerance P5CS Vigna unguiculata	GATACCATGTTCAGCCAGCTTGATGTGACTTCTTCCCAACTTCTTG TGAATGATGGATTTGCTAGGGATGCTGGCTTCAGAAAACAACTTTC GGACACAGTGAACGCGTTATTAGATTTAA	5265
Phe162Ala TTT-GCT	TTAAATCTAATAACGCGTTCACTGTGTCCGAAAGTTGTTTTCTGAA GCCAGCATCCCTA <u>GC</u> AAATCCATCATTCACAAGAAGTTGGGAAGA AGTCACATCAAGCTGGCTGAACATGGTATC	5266
	ATGGATTT <u>GC</u> TAGGGAT	5267

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
	ATCCCTAGC AAATCCAT	5268
Salt Tolerance P5CS Mesembryanthemum	GACACCTTGTTTAGTCAGTTGGATCTGACTGCTGCTCAGCTGCTT GTGACGGACAACGACGCTAGAGATCCAAGTTTTAGAACACAACTA ACTGAAACAGTGTATCAGTTGTTGGATCTAA	5269
crystallinum Phe125Ala TTT-GCT	TTAGATCCAACAACTGATACACTGTTTCAGTTAGTTGTGTTCTAAAA CTTGGATCTCTA <u>GC</u> GTCGTTGTCCGTCACAAGCAGCTGAGCAGCA GTCAGATCCAACTGACTAAACAAGGTGTC	5270
	ACAACGAC <u>GC</u> TAGAGAT	5271
	ATCTCTA <u>GC</u> GTCGTTGT	5272
Salt Tolerance P5CS Vitis vinifera	GACACATTATTTAGCCAGCTGGATGTGACATCAGCTCAGCTTCTT GTGACTGATAATGATGCTAGGGGATGAAGCTTTCCGAAATCAACTTA CTCAAACAGTGGATTCATTGTTAGCTTTGA	5273
Phe130Ala TTT-GCT	TCAAAGCTAACAATGAATCCACTGTTTGAGTAAGTTGATTTCGGAA AGCTTCATCCCTA <u>GC</u> ATCATTATCAGTCACAAGAAGCTGAGCTGAT GTCACATCCAGCTGGCTAAATAATGTGTC	5274
	ATAATGAT <u>GC</u> TAGGGAT	5275
	ATCCCTA <u>GC</u> ATCATTAT	5276
Salt Tolerance P5CS Vigna aconitifolia	GATACGCTGTTCACTCAGCTCGATGTGACATCGGCTCAGCTTCTT GTGACGGATAACGATGCTCGAGATAAGGATTTCAGGAAGCAGCTT ACTGAGACTGTGAAGTCGCTGTTGGCGCTGA	5277
Phe129Ala TTT-GCT	TCAGCGCCAACAGCGACTTCACAGTCTCAGTAAGCTGCTTCCTGA AATCCTTATCTCGAGCATCGTTATCCGTCACAAGAAGCTGAGCCG ATGTCACATCGAGCTGAGCGAACAGCGTATC	5278
	ATAACGAT <u>GC</u> TCGAGAT	5279
	ATCTCGA <u>GC</u> ATCGTTAT	5280
Salt Tolerance HKT1 Arabidopsis thaliana	AGAGATGTTCTTAGTTCCAAAGAAATCTCACCTCTCACTTTCTCCG TCTTCACAACAGTTGTCACGTTTGCAAACTGCGGATTTGTCCCCAC GAATGAGAACATGATCATCTTTCGCAAAA	5281
Ser207Val TCC-GTC	TTTTGCGAAAGATGATCATGTTCTCATTCGTGGGGACAAATCCGCA GTTTGCAAACGTG <u>AC</u> AACTGTTGTGAAGACGGAGAAAGTGAGAGG TGAGATTTCTTTGGAACTAAGAACATCTCT	5282
	CAACAGTT <u>GT</u> CACGTTT	5283
	AAACGTG <u>AC</u> AACTGTTG	5284
Satt Tolerance HKT1 <i>Arabidopsis thaliana</i>	CGAATGAGAACATGATCATCTTTCGCAAAAACTCTGGTCTCATCTG GCTCCTAATCCCTCTAGTACTGATGGGAAACACTTTGTTCCCTTGC TTCTTGGTTTTGCTCATATGGGGACTTTA	5285
Gin237Leu CAA-CTA	TAAAGTCCCCATATGAGCAAAACCAAGAAGCAAGGGAACAAAGTG TTTCCCATCAGTACTAGAGGGATTAGGAGCCAGATGAGACCAGAG TTTTTGCGAAAGATGATCATGTTCTCATTCG	5286

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	AATCCCTC <u>T</u> AGTACTGA	5287
	TCAGTACT <u>A</u> GAGGGATT	5288
Salt Tolerance HKT1	AGTCTCTAGAAGGAATGAGTTCGTACGAGAAGTTGGTTGG	5289
Arabidopsis thaliana	ACCTCTCTACACTTTCCCCAGCTATCTTGGT	
Asn332Ser AAT-AGT	ACCAAGATAGCTGGGGAAAGTGTAGAGAGGGTCTACTATAGTTTCT CCGGTGTGTCGCGAACTCACCACTTGAAACAACGATCCAACCAA	5290
	AGTGGTGAGTTCGCGAC	5291
	GTCGCGAA <u>C</u> TCACCACT	5292
Salt Tolerance HKT1 Eucalyptus	AGAGATGTGCTAAAGAAGAAAGGTCTCAAAATGGTGACCTTTTCC GTCTTCACCACCGTGGTGACCTTTGCCAGTTGTGGGTTTGTCCCG ACCAATGAAAACATGATTATCTTCAGCAAAA	5293
camaldulensis Ser256Val TCG-GTG	TTTTGCTGAAGATAATCATGTTTTCATTGGTCGGGACAAACCCACA ACTGGCAAAGGTC <u>AC</u> CACGGTGGTGAAGACGGAAAAGGTCACCA TTTTGAGACCTTTCTTCTTTAGCACATCTCT	5294
,	CCACCGTG <u>GT</u> GACCTTT	5295
	AAAGGTC <u>AC</u> CACGGTGG	5296
Salt Tolerance HKT1 Eucalyptus	CCAATGAAAACATGATTATCTTCAGCAAAAACTCTGGCCTCCTCT GATTCTCATCCCTCTGGCCCTTCTTGGGAACATGCTGTTCCCATC GAGCCTACGTTTGACGCTTTGGCTCATCGG	5297
camaldulensis Gln286Leu CAG-CTG	CCGATGAGCCAAAGCGTCAAACGTAGGCTCGATGGGAACAGCAT GTTCCCAAGAAGGGCCAGAGGGATGAGAATCAGGAGGAGGCCA GAGTTTTTGCTGAAGATAATCATGTTTTCATTGG	5298
	CATCCCTC <u>T</u> GGCCCTTC	5299
	GAAGGCCAGAGGGATG	5300
Salt Tolerance HKT1 Eucalyptus	AATCGTTGAATGGACTAAGCTCCTGTGAGAAAATCGTGGGCGCGC TGTTTCAGTGCGTGAGCAGACATACCGGCGAGACGGTCGTC GATCTGTCCACAGTTGCTCCCGCCATCTTGGT	5301
camaldulensis Asn381Ser AAC-AGC	ACCAAGATGGCGGGAGCAACTGTGGACAGATCGACGACCGTCTC GCCGGTATGTCTGCTCGCTCACGCACTGAAACAGCGCGCCCACGA TTTTCTCACAGGAGCTTAGTCCATTCAACGATT	5302
	GTGCGTGAGCAGAC	5303
	GTCTGCTGCTCACGCAC	5304

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Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ II NO:
Salt Tolerance HKT1 Oryza sativa Ser238Val	AAAGCTCCACTGAAGAAGAAAGGGATCAACATTGCACTCTTCTCAT TCTCGGTCACGGTCGTCCGTTTGCGAATGTGGGGCTCGTGCCG ACAAATGAGAACATGGCAATCTTCTCCAAGA	5305
TCC-GTC	TCTTGGAGAAGATTGCCATGTTCTCATTTGTCGGCACGAGCCCCA CATTCGCAAACGAGACGACCGTGACCGAGAATGAGAAGAGTGCA ATGTTGATCCCTTTCTTCAGTGGAGCTTT TCACGGTCGTCTCGTTT	5306
	AAACGAG <u>AC</u> GACCGTGA	5307
Salt Tolerance		5308
HKT1 Oryza sativa Gln268Leu	CAAATGAGAACATGGCAATCTTCTCCAAGAACCCGGGCCTCCTCC TCCTGTTCATCGGCCTGATTCTTGCAGGCAATACACTTTACCCTCT CTTCCTAAGGCTATTGATATGGTTCCTGGG	5309
CAG-CTG	CCCAGGAACCATATCAATAGCCTTAGGAAGAGAGGGGTAAAGTGTA TTGCCTGCAAGAATCAGGCCGATGAACAGGAGGAGGAGGCCCGG GTTCTTGGAGAAGATTGCCATGTTCTCATTTG	5310
	CATCGGCCTGATTCTTG	5311
Salt Tolerance	CAAGAATCAGGCCGATG	5312
HKT1 Oryza sativa Asn363Ser	CAGTCTTGATGGACTCAGCTCTTACCAGAAGATTATCAATGCATT GTTCATGGCAGTGAGCGCAAGGCACTCGGGGGAGAACTCCATCG ACTGCTCACTCATCGCCCCTGCTGTTCTAGT	5313
AAC-AGC	ACTAGAACAGCAGGGGCGATGAGTGAGCAGTCGATGGAGTTCTC CCCCGAGTGCCTTGCGCTCACTGCCATGAACAATGCATTGATAAT CTTCTGGTAAGAGCTGAGTCCATCAAAGACTG	5314
	GGCAGTGA <u>G</u> CGCAAGGC	5315
\=\u^2=1	GCCTTGCG <u>C</u> TCACTGCC	5316
Salt Tolerance HKT1 <i>riticum aestivum</i> Na240Val	GTGCCCCACTGAACAAGAAAGGGATCAACATCGTGCTCTTCTCAC TATCAGTCACCGTTGTCTCCTGTGCGAATGCAGGACTCGTGCCCA CAAATGAGAACATGGTCATCTTCTCAAAGAA	5317
GCC-GTC	TTCTTTGAGAAGATGACCATGTTCTCATTTGTGGGCACGAGTCCT GCATTCGCACAGGAGACAACGGTGACTGATAGTGAGAAGAGCAC GATGTTGATCCCTTTCTTGTTCAGTGGGGCAC	5318
	CACCGTTGICTCCTGTG	5319
olt Telesones	CACAGGAG <u>A</u> CAACGGTG	5320
alt Tolerance KT1 riticum aestivum In270Leu	CAAATGAGAACATGGTCATCTTCTCAAAGAATTCAGGCCTCTTGTT GCTGCTGAGTGGCCTGATGCTCGCAGGCAATACATTGTTCCCTCT CTTCCTGAGGCTACTGGTGTGGTTCCTGGG	5321
AG-CTG	CCCAGGAACCACCAGTAGCCTCAGGAAGAGAGGGAACAATGT ATTGCCTGCGAGCATCAGGCCACTCAGCAGCAACAAGAGGCCTG AATTCTTTGAGAAGATGACCATGTTCTCATTTG	5322
	GAGTGGCC <u>T</u> GATGCTCG	5323

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	CGAGCATC <u>A</u> GGCCACTC	5324
Salt Tolerance HKT1 Triticum aestivum	CAGTCTTTGATGGGCTCAGCTCTTATCAGAAGACTGTCAATGCATT CTTCATGGTGGTGAGTGCGAGGCACTCAGGGGAGAATTCCATCG ACTGCTCGCTCATGTCCCCTGCCATTATAGT	5325
Asn365Ser AAT-AGT	ACTATAATGGCAGGGGACATGAGCGAGCAGTCGATGGAATTCTCC CCTGAGTGCCTCGCACTCACCACCATGAAGAATGCATTGACAGTC TTCTGATAAGAGCTGAGCCCATCAAAGACTG	5326
	GGTGGTGA <u>G</u> TGCGAGGC	5327
	GCCTCGCACTCACCACC	5328
Freezing Tolerance proline oxidase precursor	TTTTTTTTTTCGTTTTCAAAAACAAAATCTTTGAATTTTATGGCA ACCCGTCTTCTCTGAACAAACTTTATCCGGCGATCTTACCGTTTAC CCGCTTTTAGCCCGGTGGGTCCTCCCA	5329
Arabidopsis thaliana Arg/Term CGA-TGA	TGGGAGGACCCACCGGGCTAAAAGCGGTAAACGGTAAGATCGC CGGATAAAGTTTGTTCAGAGAAGACGGGTTGCCATAAAATTCAAA GATTTTGTTTTTGAAAACGAAAAACAAAAAAAA	5330
	GTCTTCTCTGAACAAAC	5331
	GTTTGTTCAGAGAAGAC	5332
Freezing Tolerance proline oxidase precursor	TCAAAAACAAAATCTTTGAATTTTATGGCAACCCGTCTTCTCAGAA CAAACTTTATCCGGTGATCTTACCGTTTACCCGCTTTTAGCCCGGT GGGTCCTCCCACCGTGACTGCTTCCACCG	5333
Arabidopsis thaliana Arg13Term CGA-TGA	CGGTGGAAGCAGTCACGGTGGGAGGACCCACCGGGCTAAAAGC GGGTAAACGGTAAGATCACCGGATAAAGTTTGTTCTGAGAAGACG GGTTGCCATAAAATTCAAAGATTTTGTTTTTGA	5334
	TTATCCGGTGATCTTAC	5335
	GTAAGATC <u>A</u> CCGGATAA	5336
Freezing Tolerance proline oxidase precursor	AAAATCTTTGAATTTTATGGCAACCCGTCTTCTCCGAACAAACTTTA TCCGGCGATCTTAGCGTTTACCCGCTTTTAGCCCGGTGGGTCCTC CCACCGTGACTGCTTCCACCGCCGTCGTC	5337
Arabidopsis thaliana Tyr15Term TAC-TAG	GACGACGGCGGTGGAAGCAGTCACGGTGGGAGGACCCACCGGG CTAAAAGCGGGTAAACGCTAAGATCGCCGGATAAAGTTTGTTCGG AGAAGACGGGTTGCCATAAAATTCAAAGATTTT	5338
	CGATCTTAGCGTTTACC	5339
	GGTAAACGCTAAGATCG	5340

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:	
Freezing Tolerance proline oxidase precursor Arabidopsis thaliana	CTITGAATTTTATGGCAACCCGTCTTCTCCGAACAAACTTTATCCG GCGATCTTACCGTTAACCCGCTTTTAGCCCGGTGGGTCCTCCCAC CGTGACTGCTTCCACCGCCGTCGTCCCGGA		
Leu17Term TTA-TAA	TCCGGGACGACGCGGTGGAAGCAGTCACGGTGGGAGGACCCA CCGGGCTAAAAGCGGGTTAACGGTAAGATCGCCGGATAAAGTTTG TTCGGAGAAGACGGGTTGCCATAAAATTCAAAG TTACCGTTAACCCGCTT		
	AAGCGGGTTAACGGTAA	5343	
Freezing Tolerance proline oxidase precursor	CCGGTGGGTCCTCCCACCGTGACTGCTTCCACCGCCGTCGTCCC GGAGATTCTCTCTTTTGACAACAAGCACCGGAACCACCTCTTCA CCACCCAAAACCCACCGAGCAATCTCACGATG	5344	
Arabidopsis thaliana Gly42Term GGA-TGA	CATCGTGAGATTGCTCGGTGGGTTTTGGGTGGTGAAGAGGTGGT TCCGGTGCTTGTTGTCAAAAGGAGAAATCTCCGGGACGACGGC GGTGGAAGCAGTCACGGTGGGAGGACCCACCGG	5346	
	TCTCCTTT <u>T</u> GACAACAA TTGTTGTC <u>A</u> AAAGGAGA	5347	
Lead Tolerance		5348	
cyclic nucleotide- regulated ion channel	ACATGAAGCAGTGAAATCTCTGTTTGTATTGAATCTTATTAGTCTCA AACTATGAATTTCTGACAAGAGAAGTTTGTAAGGTCAGTGTTCCAG ATTTGTCTCATTGAATTCTAAGTCGTGA	5349	
Arabidopsis thaliana Arg4Term CGA-TGA	TCACGACTTAGAATTCAATGAGACAAATCTGGAACACTGACCTTAC AAACTTCTCTTGTCAGAAATTCATAGTTTGAGACTAATAAGATTCAA TACAAACAGAGATTTCACTGCTTCATGT	5350	
	TGAATTTC <u>T</u> GACAAGAG	5351	
Lood Toler	CTCTTGTC <u>A</u> GAAATTCA	5352	
Lead Tolerance cyclic nucleotide- regulated ion channel Arabidopsis thaliana	TGAAGCAGTGAAATCTCTGTTTGTATTGAATCTTATTAGTCTCAAAC TATGAATTTCCGATAAGAGAAGTTTGTAAGGTCAGTGTTCCAGATT TGTCTCATTGAATTCTAAGTCGTGAAGC	5353	
GIn5Term CAA-TAA	GCTTCACGACTTAGAATTCAATGAGACAAATCTGGAACACTGACCT TACAAACTTCTCTTATCGGAAATTCATAGTTTGAGACTAATAAGATT CAATACAAACAGAGATTTCACTGCTTCA ATTTCCGATAAGAGAAG	5354	
}	CTTCTCTTATCGGAAAT	5355	
Lead Tolerance	AGCAGTGAAATCTCTGTTTGTATTGAATCTTATTAGTCTCAAACTAT	5356	
cyclic nucleotide- regulated ion channel	GAATTTCCGACAA <u>T</u> AGAAGTTTGTAAGGTCAGTGTTCCAGATTTGT CTCATTGAATTCTAAGTCGTGAAGCTTA	5357	
Glu6Term	TAAGCTTCACGACTTAGAATTCAATGAGACAAATCTGGAACACTGA CCTTACAAACTTCTATTGTCGGAAATTCATAGTTTGAGACTAATAA GATTCAATACAAACAGAGATTTCACTGCT	5358	

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	TCCGACAA <u>T</u> AGAAGTTT	5359
	AAACTTCT <u>A</u> TTGTCGGA	5360
Lead Tolerance cyclic nucleotide-regulated ion channel	AGTGAAATCTCTGTTTGTATTGAATCTTATTAGTCTCAAACTATGAA TTTCCGACAAGAGTAGTTTGTAAGGTCAGTGTTCCAGATTTGTCTC ATTGAATTCTAAGTCGTGAAGCTTAATT	5361
Arabidopsis thaliana Lys7Term AAG-TAG	AATTAAGCTTCACGACTTAGAATTCAATGAGACAAATCTGGAACAC TGACCTTACAAACTACTCTTGTCGGAAATTCATAGTTTGAGACTAA TAAGATTCAATACAAACAGAGATTTCACT	5362
MOTINO	GACAAGAG <u>T</u> AGTTTGTA	5363
	TACAAACT <u>A</u> CTCTTGTC	5364
Lead Tolerance cyclic nucleotide- regulated ion channel	CATTGAATTCTAAGTCGTGAAGCTTAATTCGATTCTTCTTCACTTTC TCGGATCAGGTTTTAAGATTGGAAGTCGGATAAGACTTCCTCCGA CGTGGAATATTCCGGTAAAAAACGAGATTC	5365
Arabidopsis thaliana Gln12Term CAA-TAA	GAATCTCGTTTTTACCGGAATATTCCACGTCGGAGGAAGTCTTATC CGACTTCCAATCTTAAAACCTGATCCGAGAAAGTGAAGAAGAATC GAATTAAGCTTCACGACTTAGAATTCAATG	5366
CAN-IAN	TCAGGTTTAAGATTGG	5367
	CCAATCTTAAAACCTGA	5368
Lead Tolerance cyclic nucleotide-	TGGAAGTCAATCCCCCACGTTGAGCAGGTTGATGCATTGGCTAAA GTTATGAATCACCGCTAAGACGAGTTTGTGAGGTTTCAGGATTGG AAATCAGAGAGAAGCTCTGAGGGAAATTTTC	5369
gated calmodulin- binding ion channel (CBP4) Nicotiana Tabacum	GAAAATTTCCCTCAGAGCTTCTCTCTGATTTCCAATCCTGAAACCT CACAAACTCGTCTTAGCGGTGATTCATAACTTTAGCCAATGCATCA ACCTGCTCAACGTGGGGGATTGACTTCCA	5370
GIn5Term	ATCACCGCTAAGACGAG	5371
CAA-TAA	CTCGTCTTAGCGGTGAT	5372
Lead Tolerance cyclic nucleotide- gated calmodulin- binding ion channel (CBP4) Nicotiana Tabacum Gly7Term	TCAATCCCCCACGTTGAGCAGGTTGATGCATTGGCTAAAGTTATG AATCACCGCCAAGACTAGTTTGTGAGGTTTCAGGATTGGAAATCA GAGAGAAGCTCTGAGGGAAATTTTCATGCTA	5373
	TAGCATGAAAATTTCCCTCAGAGCTTCTCTCTGATTTCCAATCCTG AAACCTCACAAACTAGTCTTGGCGGTGATTCATAACTTTAGCCAAT GCATCAACCTGCTCAACGTGGGGGGATTGA	5374
	GCCAAGACTAGTTTGTG	5375
GAG-TAG	CACAAACTAGTCTTGGC	5376

	Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID NO:
5	Lead Tolerance cyclic nucleotide- gated calmodulin- binding ion channel (CBP4) Nicotiana Tabacum GIn12Term CAG-TAG	GAGCAGGTTGATGCATTGGCTAAAGTTATGAATCACCGCCAAGAC GAGTTTGTGAGGTTTTAGGATTGGAAATCAGAGAGAAGCTCTGAG GGAAATTTTCATGCTAAAGGTGGAGTCCACC	5377
		GGTGGACTCCACCTTTAGCATGAAAATTTCCCTCAGAGCTTCTCTC TGATTTCCAATCCTAAAACCTCACAAACTCGTCTTGGCGGTGATTC ATAACTTTAGCCAATGCATCAACCTGCTC	5378
		TGAGGTTT <u>T</u> AGGATTGG	5379
		CCAATCCT <u>A</u> AAACCTCA	5380
10	Lead Tolerance cyclic nucleotide- gated calmodulin-	TGATGCATTGGCTAAAGTTATGAATCACCGCCAAGACGAGTTTGT GAGGTTTCAGGATTGTAAATCAGAGAGAAGCTCTGAGGGAAATTT TCATGCTAAAGGTGGAGTCCACCGAAGTAAA	5381
15	binding ion channel (CBP4) Nicotiana Tabacum	TITACTTCGGTGGACTCCACCTTTAGCATGAAAATTTCCCTCAGAG CTTCTCTCTGATTTACAATCCTGAAACCTCACAAACTCGTCTTGGC GGTGATTCATAACTTTAGCCAATGCATCA	5382
וט	Trp14Term TGG-TGA	CAGGATTG <u>T</u> AAATCAGA	5383
		TCTGATTT <u>A</u> CAATCCTG	5384
20	Lead Tolerance cyclic nucleotide- gated calmodulin- binding ion channel (CBP4) Nicotiana Tabacum Lys15Term AAA-TAA	GATGCATTGGCTAAAGTTATGAATCACCGCCAAGACGAGTTTGTG AGGTTTCAGGATTGGTAATCAGAGAAGACTCTGAGGGAAATTTT CATGCTAAAGGTGGAGTCCACCGAAGTAAAG	5385
		CTITACTTCGGTGGACTCCACCTTTAGCATGAAAATTTCCCTCAGA GCTTCTCTGATTACCAATCCTGAAACCTCACAAACTCGTCTTGG CGGTGATTCATAACTTTAGCCAATGCATC	5386
		AGGATTGG <u>T</u> AATCAGAG	5387
		CTCTGATT <u>A</u> CCAATCCT	5388
25 30	Lead Tolerance calmodulin binding transport protein Hordeum vulgare Glu2Term GAA-TAA	CTTGAAGAATTGATCTACCACTCTTAGCTGCTAACTGTTCGCCTGG TGGAGATAATGATGTAAAGAGAGACAGATATGTTAGATTTCAGGA CTGCAAATCAGAGCAATCTGTTATCTCAG	5389
		CTGAGATAACAGATTGCTCTGATTTGCAGTCCTGAAATCTAACATA TCTGTCCTCTCTTACATCATTATCTCCACCAGGCGAACAGTTAGC AGCTAAGAGTGGTAGATCAATTCTTCAAG	5390
		TAATGATG <u>T</u> AAAGAGAG	5391
		CTCTCTTT <u>A</u> CATCATTA	5392

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Lead Tolerance calmodulin binding transport protein	GAAGAATTGATCTACCACTCTTAGCTGCTAACTGTTCGCCTGGTG GAGATAATGATGGAATGAGAGGACAGATATGTTAGATTTCAGGAC TGCAAATCAGAGCAATCTGTTATCTCAGAGA	5393
Hordeum vulgare Arg3Term AGA-TGA	TCTCTGAGATAACAGATTGCTCTGATTTGCAGTCCTGAAATCTAAC ATATCTGTCCTCCATTCCAT	5394
	TGATGGAA <u>T</u> GAGAGGAC	5395
	GTCCTCTC <u>A</u> TTCCATCA	5396
Lead Tolerance calmodulin binding transport protein	GAATTGATCTACCACTCTTAGCTGCTAACTGTTCGCCTGGTGGAG ATAATGATGGAAAGA <u>T</u> AGGACAGATATGTTAGATTTCAGGACTGCA AATCAGAGCAATCTGTTATCTCAGAGAACG	5397
Hordeum vulgare Glu4Term GAG-TAG	CGTTCTCTGAGATAACAGATTGCTCTGATTTGCAGTCCTGAAATCT AACATATCTGTCCTATCTTTCCATCATTATCTCCACCAGGCGAACA GTTAGCAGCTAAGAGTGGTAGATCAATTC	5398
	TGGAAAGA <u>T</u> AGGACAGA	5399
	TCTGTCCT <u>A</u> TCTTTCCA	5400
Lead Tolerance calmodulin binding transport protein	ATCTACCACTCTTAGCTGCTAACTGTTCGCCTGGTGGAGATAATG ATGGAAAGAGGGACTGATATGTTAGATTTCAGGACTGCAAATCA GAGCAATCTGTTATCTCAGAGAACGCAGTTT	5401
Hordeum vulgare Arg6Term AGA-TGA	AAACTGCGTTCTCTGAGATAACAGATTGCTCTGATTTGCAGTCCTG AAATCTAACATATCAGTCCTCTCTTTCCATCATTATCTCCACCAGG CGAACAGTTAGCAGCTAAGAGTGGTAGAT	5402
	GAGAGGAC <u>T</u> GATATGTT	5403
	AACATATC <u>A</u> GTCCTCTC	5404
Lead Tolerance calmodulin binding transport protein	CCACTCTTAGCTGCTAACTGTTCGCCTGGTGGAGATAATGATGGA AAGAGAGGACAGATAGGTTAGATTTCAGGACTGCAAATCAGAGCA ATCTGTTATCTCAGAGAACGCAGTTTCACCA	5405
Hordeum vulgare Tyr7Term TAT-TAG	TGGTGAAACTGCGTTCTCTGAGATAACAGATTGCTCTGATTTGCAG TCCTGAAATCTAACCTATCTGTCCTCTTTTCCATCATTATCTCCAC CAGGCGAACAGTTAGCAGCTAAGAGTGG	5406
	GACAGATA <u>G</u> GTTAGATT	5407
	AATCTAAC <u>C</u> TATCTGTC	5408
2,4-DB resistance 3-ketoacyl-CoA thiolase	ATCCTTCTCGAGAAAAAACAACAGATCCGAATTTTATCTTTAATCA GCCGGAAAAAATGTAGAAAGCGATCGAGAGACAACGCGTTCTTCT TGAGCATCTCCGACCTTCTTCTTCTTCTT	5409
Arabidopsis thaliana Glu2Term GAG-TAG	AAGAAGAAGAAGAAGGTCGGAGATGCTCAAGAAGAACGCGTTGTC TCTCGATCGCTTTCTACATTTTTTCCGGCTGATTAAAGATAAAATTC GGATCTGTTGTTTTTTCTCAGAGAAGGAT	5410

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID
	AAAAAATG <u>T</u> AGAAAGCG	5411
	CGCTTTCT <u>A</u> CATTTTTT	5412
2,4-DB resistance 3-ketoacyl-CoA thiolase	CTTCTCTGAGAAAAAACAACAGATCCGAATTTTATCTTTAATCAGC CGGAAAAAATGGAGTAAGCGATCGAGAGACAACGCGTTCTTCTTG AGCATCTCCGACCTTCTTCTTCTTCGC	5413
Arabidopsis thaliana Lys3Term AAA-TAA	GCGAAGAAGAAGAAGAAGGTCGGAGATGCTCAAGAAGAACGCGT TGTCTCTCGATCGCTTACTCCATTTTTTCCGGCTGATTAAAGATAA AATTCGGATCTGTTGTTTTTTCTCAGAGAAG	5414
	AAATGGAG <u>T</u> AAGCGATC	5415
	GATCGCTTACTCCATTT	5416
2,4-DB resistance 3-ketoacyl-CoA thiolase	GAAAAAACAACAGATCCGAATTTTATCTTTAATCAGCCGGAAAAAA TGGAGAAAGCGATCTAGAGACAACGCGTTCTTCTTGAGCATCTCC GACCTTCTTCTTCTTCGCACAATTACG	5417
Arabidopsis thaliana Glu6Term GAG-TAG	CGTAATTGTGCGAAGAAGAAGAAGAAGGTCGGAGATGCTCAAGAA GAACGCGTTGTCTCTAGATCGCTTTCTCCATTTTTTCCGGCTGATT AAAGATAAAATTCGGATCTGTTGTTTTTTC	5418
	AAGCGATC <u>T</u> AGAGACAA	5419
	TTGTCTCTAGATCGCTT	5420
2,4-DB resistance 3-ketoacyl-CoA thiolase	AAAACAACAGATCCGAATTTTATCTTTAATCAGCCGGAAAAAATGG AGAAAGCGATCGAGTGACAACGCGTTCTTCTTGAGCATCTCCGAC CTTCTTCTTCTTCGCACAATTACGAGG	5421
Arabidopsis thaliana Arg7Term AGA-TGA	CCTCGTAATTGTGCGAAGAAGAAGAAGAAGGTCGGAGATGCTCAA GAAGAACGCGTTGTCACTCGATCGCTTTCTCCATTTTTTCCGGCT GATTAAAGATAAAATTCGGATCTGTTGTTTT	5422
	CGATCGAG <u>T</u> GACAACGC	5423
	GCGTTGTC <u>A</u> CTCGATCG	5424
2,4-DB resistance 3-ketoacyl-CoA thiolase	ACAACAGATCCGAATTTTATCTTTAATCAGCCGGAAAAAATGGAGA AAGCGATCGAGAGA <u>T</u> AACGCGTTCTTCTTGAGCATCTCCGACCTT CTTCTTCTTCGCACAATTACGAGGCTT	5425
Arabidopsis thaliana Gln8Term CAA-TAA	AAGCCTCGTAATTGTGCGAAGAAGAAGAAGAAGAAGGTCGGAGATGCT CAAGAAGAACGCGTTATCTCTCGATCGCTTTCTCCATTTTTTCCGG CTGATTAAAGATAAAATTCGGATCTGTTGT	5426
	TCGAGAGA <u>T</u> AACGCGTT	5427
	AACGCGTT <u>A</u> TCTCTCGA	5428

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Phenotype, Gene, Plant & Targeted Atteration	Attering Oligos	SEQ NO
2,4-DB resistance glyoxysomal beta- ketoacyol-thiolase	GAGAGACAAAGAGTTCTTCTTGAACATCTCCGTCCTTCTTCTTCTTCTTCTCTCTC	542
precursor  Brassica napus Glu26Term	AGGTCCTCTGATACGCAGCACTGTCCCCAGCCAAGCAAGC	543
GAA-TAA	ACAGCTTT <u>T</u> AAGGCTCT	543
	AGAGCCTT <u>A</u> AAAGCTGT	543
2,4-DB resistance glyoxysomal beta- ketoacyol-thiolase	TTGAACATCTCCGTCCTTCTTCTTCTTCTCTCACAGCTTTGAAGG CTCTCTCTCTGCTTGAGCTTGCTTGGCTGGGGACAGTGCTGCGTA TCAGAGGACCTCTCTCTATGGAGATGATGT	543
precursor  Brassica napus Ser32Term	ACATCATCTCCATAGAGAGAGGTCCTCTGATACGCAGCACTGTCC CCAGCCAAGCAAGCTCAAGCAGAGAGAGAGCCTTCAAAGCTGTG AGAGGAAGAAGAAGAAGGACGGAGATGTTCAA	543
TCA-TGA	CTCTGCTT <u>G</u> AGCTTGCT	543
	AGCAAGCT <u>C</u> AAGCAGAG	543
2,4-DB resistance glyoxysomal beta- ketoacyol-thiolase	TCTCCGTCCTTCTTCTTCTTCCTCACAGCTTTGAAGGCTCTCTC TCTGCTTCAGCTTGATTGGCTGGGGACAGTGCTGCGTATCAGAG GACCTCTCTCTATGGAGATGATGTAGTCATT	543
precursor  Brassica napus  Cys34Term	AATGACTACATCATCTCCATAGAGAGAGGTCCTCTGATACGCAGC ACTGTCCCCAGCCAATCAAGCTGAAGCAGAGAGAGAGCCTTCAAA GCTGTGAGAGAAGAAGAAGAAGGACGGAGA	543
TGC-TGA	TCAGCTTGATTGGCTGG	543
	CCAGCCAA <u>T</u> CAAGCTGA	544
2,4-DB resistance glyoxysomal beta- ketoacyol-thiolase precursor Brassica napus Leu35Term	TCCGTCCTTCTTCTTCCTCTCACAGCTTTGAAGGCTCTCTCT	544
	ACAATGACTACATCATCTCCATAGAGAGAGGTCCTCTGATACGCA GCACTGTCCCCAGCCTAGCAAGCTGAAGCAGAGAGAGAGCCTTC AAAGCTGTGAGAGAAGAAGAAGAAGAAGAAGAAGAA	544
TTG-TAG	AGCTTGCTAGGCTGGGG	544
	CCCCAGCCTAGCAAGCT	544

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
2,4-DB resistance glyoxysomal beta- ketoacyol-thiolase	TCACAGCTTTGAAGGCTCTCTCTCTCTCTCAGCTTGCTTG	5445
precursor Brassica napus Tyr42Term TAT-TAG	TAGTGCAGTCCTATGTGCCGCAACAATGACTACATCATCTCCATA GAGAGAGGTCCTCTGCTACGCAGCACTGTCCCCAGCCAAGCAAG	5446
	GCTGCGTAGCAGGAC GTCCTCTGCTACGCAGC	5447
2,4-DB resistance		5448
3-ketoacyl-CoA thiolase B	CAACAGACAGCAAGTGTTGCTCCAGCATCTCCGCCCTTCTAATTC TTCTTCTCACAATTAGGAGTCCGCTCTTGCCGCATCAGTATGTGCT GCAGGGGATAGCGCCGCATATCATAGGGCT	5449
Mangifera indica Tyr25Term TAC-TAG	AGCCCTATGATATGCGGCGCTATCCCCTGCAGCACATACTGATGC GGCAAGAGCGGACTCCTAATTGTGAGAAGAAGAATTAGAAGGGC GGAGATGCTGGAGCAACACTTGCTGTTGTTG	5450
	CACAATTA <u>G</u> GAGTCCGC	5451
	GCGGACTC <u>C</u> TAATTGTG	5452
2,4-DB resistance 3-ketoacyol-CoA thiolase B	AACAGACAGCAAGTGTTGCTCCAGCATCTCCGCCCTTCTAATTCTT CTTCTCACAATTACTAGTCCGCTCTTGCCGCATCAGTATGTGCTG CAGGGGATAGCGCCGCATATCATAGGGCTT	5453
Magnifera indica Glu26Term GAG-TAG	AAGCCCTATGATATGCGGCGCTATCCCCTGCAGCACATACTGATG CGGCAAGAGCGGACTAGTAATTGTGAGAAGAAGAATTAGAAGGG CGGAGATGCTGGAGCAACACTTGCTGTCTGTT	5454
	ACAATTAC <u>T</u> AGTCCGCT	5455
	AGCGGACT <u>A</u> GTAATTGT	5456
2,4-DB resistance 3-ketoacytol-CoA thiolase B	TCCAGCATCTCCGCCCTTCTAATTCTTCTCTCACAATTACGAGTC CGCTCTTGCCGCATGAGTATGTGCTGCAGGGGGATAGCGCCGCAT ATCATAGGGCTTCTGTTTATGGAGACGATGT	5457
<i>Mangifera indica</i> Ser32Term FCA-TGA	ACATCGTCTCCATAAACAGAAGCCCTATGATATGCGGCGCTATCC CCTGCAGCACATACTCATGCGGCAAGAGCGGACTCGTAATTGTGA GAAGAAGAATTAGAAGGGCGGAGATGCTGGA	5458
	TGCCGCAT <u>G</u> AGTATGTG	5459
	CACATACT <u>C</u> ATGCGGCA	5460

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
2,4-DB resistance 3-ketoacyl-CoA thiolase B	TCTCCGCCCTTCTAATTCTTCTTCTCACAATTACGAGTCCGCTCTT GCCGCATCAGTATGAGCTGCAGGGGGATAGCGCCGCATATCATAG GGCTTCTGTTTATGGAGACGATGTGGTGATT	5461
Mangifera indica Cys34Term TGT-TGA	AATCACCACATCGTCTCCATAAACAGAAGCCCTATGATATGCGGC GCTATCCCCTGCAGCTCATACTGATGCGGCAAGAGCGGACTCGT AATTGTGAGAAGAAGAATTAGAAGGGCGGAGA	5462
10111011	TCAGTATGAGCTGCAGG	5463
	CCTGCAGC <u>T</u> CATACTGA	5464
2,4-DB resistance 3-ketoacyl-CoA thiolase B	TCACAATTACGAGTCCGCTCTTGCCGCATCAGTATGTGCTGCAGG GGATAGCGCCGCATAGCATA	5465
Mangifera indica Tyr42Term TAT-TAG	AAGTGCAGTACGATGAGCTGCCACAATCACCACATCGTCTCCATA AACAGAAGCCCTATGCTATG	5466
7.11 17.0	GCCGCATAGCATAGGGC	5467
	GCCCTATGCTATGCGGC	5468
2,4-DB resistance 3-ketoacyl-CoA thiolase	GAAGGCGATCAACAGGCAGAGCATTTTGCTACATCATCTCCGGCC TTCTTCTTCCGCTTAGACAAATGAATCTTCGCTCTCTGCATCGGTT TGTGCAGCTGGGGATAGTGCTTCGTATCAA	5469
Cucumis sativus Tyr22Term TAC-TAG	TTGATACGAAGCACTATCCCCAGCTGCACAAACCGATGCAGAGAG CGAAGATTCATTTGTCTAAGCGGAAGAAGAAGGCCGGAGATGATG TAGCAAAATGCTCTGCCTGTTGATCGCCTTC	5470
IAO-IAO	TCCGCTTAGACAAATGA	5471
	TCATTTGTCTAAGCGGA	5472
2,4-DB resistance 3-ketoacyl-CoA thiolase	ATCAACAGGCAGAGCATTTTGCTACATCATCTCCGGCCTTCTTCTT CCGCTTACACAAATTAATCTTCGCTCTCTGCATCGGTTTGTGCAGC TGGGGATAGTGCTTCGTATCAAAGGACAT	5473
Cucumis sativus Glu25Term GAA-TAA	ATGTCCTTTGATACGAAGCACTATCCCCAGCTGCACAAACCGATG CAGAGAGCGAAGATTAATTTGTGTAAGCGGAAGAAGAAGGCCGG AGATGATGTAGCAAAATGCTCTGCCTGTTGAT	5474
	ACACAAATTAATCTTCG	5475
	CGAAGATTAATTTGTGT	5476

Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID NO:
2,4-DB resistance 3-ketoacyl-CoA thiolase	GGCAGAGCATTTTGCTACATCATCTCCGGCCTTCTTCTTCCGCTTA CACAAATGAATCTTAGCTCTCTGCATCGGTTTGTGCAGCTGGGGA TAGTGCTTCGTATCAAAGGACATCGGTGTT	5477
Cucumis sativus Ser27Term TCG-TAG	AACACCGATGTCCTTTGATACGAAGCACTATCCCCAGCTGCACAA ACCGATGCAGAGAGCTAAGATTCATTTGTGTAAGCGGAAGAAGAA GGCCGGAGATGATGTAGCAAAATGCTCTGCC	5478
	TGAATCTTAGCTCTCTG	5479
	CAGAGAGC <u>T</u> AAGATTCA	5480
2,4-DB resistance 3-ketoacyl-CoA thiolase	TGCTACATCATCTCCGGCCTTCTTCTTCCGCTTACACAAATGAATC TTCGCTCTCTGCATAGGTTTGTGCAGCTGGGGATAGTGCTTCGTA TCAAAGGACATCGGTGTTTGGAGATGATGT	5481
Cucumis sativus Ser31Term TCG-TAG	ACATCATCTCCAAACACCGATGTCCTTTGATACGAAGCACTATCCC CAGCTGCACAAACCTATGCAGAGAGCGAAGATTCATTTGTGTAAG CGGAAGAAGAAGGCCGGAGATGATGTAGCA	5482
	CTCTGCAT <u>A</u> GGTTTGTG	5483
	CACAAACC <u>T</u> ATGCAGAG	5484
2,4-DB resistance 3-ketoacyl-CoA thiolase	TCATCTCCGGCCTTCTTCTCCGCTTACACAAATGAATCTTCGCTC TCTGCATCGGTTTGAGCAGCTGGGGGATAGTGCTTCGTATCAAAGG ACATCGGTGTTTGGAGATGATGTCGTGATT	5485
Cucumis sativus Cys33Term TGT-TGA	AATCACGACATCATCTCCAAACACCGATGTCCTTTGATACGAAGCA CTATCCCCAGCTGCTCAAACCGATGCAGAGAGCGAAGATTCATTT GTGTAAGCGGAAGAAGAAGGCCGGAGATGA	5486
	TCGGTTTGAGCAGCTGG	5487
	CCAGCTGC <u>T</u> CAAACCGA	5488
2,4-DB resistance 3-ketoacyl-CoA thiolase	GAAGGCAATCAACAGGCAGAGCATTCTGCTACATCATCTCCGGCC TTCATCTTCGGCTTAGAGCCATGAATCTTCGCTCTCTGCATCGGTT TGTGCAGCTGGGGATAGTGCGTCGTATCAA	5489
Cucurbita sp. Tyr22Term TAT-TAG	TTGATACGACGCACTATCCCCAGCTGCACAAACCGATGCAGAGAG CGAAGATTCATGGCTCTAAGCCGAAGATGAAGGCCGGAGATGAT GTAGCAGAATGCTCTGCCTGTTGATTGCCTTC	5490
	TCGGCTTA <u>G</u> AGCCATGA	5491
	TCATGGCT <u>C</u> TAAGCCGA	5492

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ III
2,4-DB resistance 3-ketoacyl-CoA thiolase	ATCAACAGGCAGAGCATTCTGCTACATCATCTCCGGCCTTCATCTT CGGCTTATAGCCATTAATCTTCGCTCTCTGCATCGGTTTGTGCAG CTGGGGATAGTGCGTCGTATCAAAGAACGT	5493
Cucurbita sp. Glu25Term GAA-TAA	ACGTTCTTTGATACGACGCACTATCCCCAGCTGCACAAACCGATG CAGAGAGCGAAGATTAATGGCTATAAGCCGAAGATGAAGGCCGG AGATGATGTAGCAGAATGCTCTGCCTGTTGAT	5494
OAA IAA	ATAGCCAT <u>T</u> AATCTTCG	5495
	CGAAGATT <u>A</u> ATGGCTAT	5496
2,4-DB resistance 3-ketoacyl-CoA thiolase	GGCAGAGCATTCTGCTACATCATCTCCGGCCTTCATCTTCGGCTT ATAGCCATGAATCTTAGCTCTCTGCATCGGTTTGTGCAGCTGGGG ATAGTGCGTCGTATCAAAGAACGTCGGTGTT	5497
Cucurbita sp. Ser27Term TCG-TAG	AACACCGACGTTCTTTGATACGACGCACTATCCCCAGCTGCACAA ACCGATGCAGAGAGCTAAGATTCATGGCTATAAGCCGAAGATGAA GGCCGGAGATGATGTAGCAGAATGCTCTGCC	5498
,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	TGAATCTTAGCTCTCTG	549
	CAGAGAGCTAAGATTCA	550
2,4-DB resistance 3-ketoacyl-CoA thiolase	TGCTACATCATCTCCGGCCTTCATCTTCGGCTTATAGCCATGAATC TTCGCTCTCTGCATAGGTTTGTGCAGCTGGGGATAGTGCGTCGTA TCAAAGAACGTCGGTGTTTGGAGATGATGT	550
Cucurbita sp. Ser31Term TCG-TAG	ACATCATCTCCAAACACCGACGTTCTTTGATACGACGCACTATCCC CAGCTGCACAAACCTATGCAGAGAGCGAAGATTCATGGCTATAAG CCGAAGATGAAGGCCGGAGATGATGTAGCA	550
100-170	CTCTGCATAGGTTTGTG	550
•	CACAAACC <u>T</u> ATGCAGAG	550
2,4-DB resistance 3-ketoacyl-CoA	TCATCTCCGGCCTTCATCTTCGGCTTATAGCCATGAATCTTCGCTC TCTGCATCGGTTTGAGCAGCTGGGGATAGTGCGTCGTATCAAAGA ACGTCGGTGTTTGGAGATGATGTCGTGATA	550
thiolase Cucurbita sp. Cys33Term	TATCACGACATCATCTCCAAACACCGACGTTCTTTGATACGACGCA CTATCCCCAGCTGCTCAAACCGATGCAGAGAGCGAAGATTCATGG CTATAAGCCGAAGATGAAGGCCGGAGATGA	550
TGT-TGA	TCGGTTTGAGCAGCTGG	550
	CCAGCTGCTCAAACCGA	550
2,4 DB resistance Pex14 Arabidopsis thaliana	TCATAGTCTCTTTTGCCGCTTGGATTCTTCCAAGGTTAGTGAGCTG CTATGGCAACTCATTAGCAAACGCAACCTCCTTCCGATTTTCCCG CTCTTGCCGATGAAAATTCCCAGATTCCAG	550
GIn5Term CAG-TAG	CTGGAATCTGGGAATTTCATCGGCAAGAGCGGGAAAATCGGAAG GAGGTTGCGTTTGCTAATGAGTTGCCATAGCAGCTCACTAACCTT GGAAGAATCCAAGCGGCAAAAGAGACTATGA	551

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	CAACTCATTAGCAAACG	5511
	CGTTTGCTAATGAGTTG	5512
2,4 DB resistance Pex14 Arabidopsis thaliana	TAGTCTCTTTTGCCGCTTGGATTCTTCCAAGGTTAGTGAGCTGCTA TGGCAACTCATCAGTAAACGCAACCTCCTTCCGATTTTCCCGCTC TTGCCGATGAAAATTCCCAGATTCCAGGTT	5513
Gin6Term CAA-TAA	AACCTGGAATCTGGGAATTTTCATCGGCAAGAGCGGGAAAATCGG AAGGAGGTTGCGTTTACTGATGAGTTGCCATAGCAGCTCACTAAC CTTGGAAGAATCCAAGCGGCAAAAGAGACTA	5514
	CTCATCAGTAAACGCAA	5515
	TTGCGTTT <u>A</u> CTGATGAG	5516
2,4 DB resistance Pex14 Arabidopsis thaliana	CTTTTGCCGCTTGGATTCTTCCAAGGTTAGTGAGCTGCTATGGCA ACTCATCAGCAAACGTAACCTCCTTCCGATTTTCCCGCTCTTGCC GATGAAAATTCCCAGATTCCAGGTTCAATTT	5517
GIn8Term CAA-TAA	AAATTGAACCTGGAATCTGGGAATTTTCATCGGCAAGAGCGGGAA AATCGGAAGGAGGTTACGTTTGCTGATGAGTTGCCATAGCAGCTC ACTAACCTTGGAAGAATCCAAGCGGCAAAAG	5518
	AGCAAACG <u>T</u> AACCTCCT	5519
	AGGAGGTT <u>A</u> CGTTTGCT	5520
2,4 DB resistance Pex14 Arabidopsis thaliana	GCTGCTATGGCAACTCATCAGCAAACGCAACCTCCTTCCGATTTT CCCGCTCTTGCCGATTAAAATTCCCAGATTCCAGGTTCAATTTACA CCTTCTAATCATTATTTCTTAATTTTTCTT	5521
Glu19Term GAA-TAA	AAGAAAAATTAAGAAATAATGATTAGAAGGTGTAAATTGAACCTGG AATCTGGGAATTTT <u>A</u> ATCGGCAAGAGCGGGAAAAATCGGAAGGAG GTTGCGTTTGCTGATGAGTTGCCATAGCAGC	5522
	TTGCCGATTAAAATTCC	5523
	GGAATTIT <u>A</u> ATCGGCAA	5524
2,4 DB resistance Pex14 Arabidopsis thaliana	GCAACTCATCAGCAAACGCAACCTCCTTCCGATTTTCCCGCTCTT GCCGATGAAAATTCCTAGATTCCAGGTTCAATTTACACCTTCTAAT CATTATTTCTTAATTTTTCTTTGGTGGATT	5525
Gin22Term CAG-TAG	AATCCACCAAAGAAAAATTAAGAAATAATGATTAGAAGGTGTAAATT GAACCTGGAATCTAGGAATTTTCATCGGCAAGAGCGGGAAAATCG GAAGGAGGTTGCGTTTGCTGATGAGTTGC	5526
	AAAATTCC <u>T</u> AGATTCCA	5527
	TGGAATCT <u>A</u> GGAATTTT	5528

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## Example 8

# Production of albino mutants for the analysis of photosynthetic processes

Plant productivity is limited by resources available and the ability of plants to harness these resources. The conversion of light to chemical energy, which is then used to synthesize carbohydrates, fatty acids, sugars, amino acids and other compounds, requires a complex system which combines the light harvesting apparatus of pigments and proteins. The value of light energy to the plant can only be realized when it is efficiently converted into chemical energy by photosynthesis and fed into various biochemical processes. Significant effort has therefore been directed at studying photosynthetic processes in plants in order to improve productivity and/or the efficiency of photosynthesis. The analysis of the photosynthetic process is substantially aided by the ability to produce albino plants.

The attached table discloses exemplary oligonucleotide base sequences which can be used to generate site-specific mutations in genes involved in starch metabolism.

Table 18
Oligonucleotides to produce albino plants

Phenotype, Gene, Plant & Targeted Alteration	Aftering Oligos	SEQID NO:
White leaves Immutans Arabidopsis thaliana	TTCTTTCCTGTGAAATTATCTGCTCAAATCTTTGGTTCCTGACGGAG ATGGCGGCGATTTGAGGCATCTCCTCTGGTACGTTGACGATTTCA CGGCCTTTGGTTACTCTTCGACGCTCTAG	
Ser5Term TCA-TGA	CTAGAGCGTCGAAGAGTAACCAAAGGCCGTGAAATCGTCAACGTA CCAGAGGAGATGCCT <u>C</u> AAATCGCCGCCATCTCCGTCAGGAACCAA AGATTTGAGCAGATAATTTCACAGGAAAGAA	5530
	GGCGATTT <u>G</u> AGGCATCT	5531
	AGATGCCT <u>C</u> AAATCGCC	5532
White leaves Immutans Arabidopsis thaliana	GCTCAAATCTTTGGTTCCTGACGGAGATGGCGGCGATTTCAGGCA TCTCCTCTGGTACGTAGACGATTTCACGGCCTTTGGTTACTCTTCG ACGCTCTAGAGCCGCCGTTTCGTACAGCTC	5533
Leu12Tem TTG-TAG	GAGCTGTACGAAACGGCGGCTCTAGAGCGTCGAAGAGTAACCAAA GGCCGTGAAATCGTCTACGTACCAGAGGAGATGCCTGAAATCGCC GCCATCTCCGTCAGGAACCAAAGATTTGAGC	5534
	TGGTACGT <u>A</u> GACGATTT	5535
	AAATCGTC <u>T</u> ACGTACCA	5536
White leaves Immutans <i>Arabidopsis thaliana</i>	TTTGGTTCCTGACGGAGATGGCGGCGATTTCAGGCATCTCCTCTG GTACGTTGACGATTTGACGCCTTTGGTTACTCTTCGACGCTCTAG AGCCGCCGTTTCGTACAGCTCCTCTCACCG	5537
Ser15Term TCA-TGA	CGGTGAGAGGAGCTGTACGAAACGGCGGCTCTAGAGCGTCGAAG AGTAACCAAAGGCCGTCAAATCGTCAACGTACCAGAGGAGATGCC TGAAATCGCCGCCATCTCCGTCAGGAACCAAA	5538
	GACGATTT <u>G</u> ACGGCCTT	5539
	AAGGCCGT <u>C</u> AAATCGTC	5540
White leaves Immutans Arabidopsis thaliana	GCGCCGATTTCAGGCATCTCCTCTGGTACGTTGACGATTTCACGG CCTTTGGTTACTCTTTGACGCTCTAGAGCCGCCGTTTCGTACAGCT CCTCTCACCGATTGCTTCATCATCTTCCTC	5541
Arg22Term CGA-TGA	GAGGAAGATGATGAAGCAATCGGTGAGAGGAGCTGTACGAAACG GCGGCTCTAGAGCGTCAAAGAGTAACCAAAGGCCGTGAAATCGTC AACGTACCAGAGGAGATGCCTGAAATCGCCGC	5542
	TTACTCTTTGACGCTCT	5543
	AGAGCGTCAAAGAGTAA	5544

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White leaves	TCAGGCATCTCCTCTGGTACGTTGACGATTTCACGGCCTTTGGTTA	5545
Immutans ·	CTCTTCGACGCTCTTGAGCCGCCGTTTCGTACAGCTCCTCTCACC	
Arabidopsis thaliana	GATTGCTTCATCATCTTCCTCTCTCTCTC	
Arg25Term	GAGAAGAGAGAAGATGATGAAGCAATCGGTGAGAGGAGCTG	5546
AĞA-TGA	TACGAAACGCCGCTCAAGAGCGTCGAAGAGTAACCAAAGGCCG	
	TGAAATCGTCAACGTACCAGAGGAGATGCCTGA	
	GACGCTCTTGAGCCGCC	5547
	GGCGGCTC <u>A</u> AGAGCGTC	5548
White leaves	GATTCTTGTGGGAAGGAAGAAGGATCAAGAATGGCGATTTCGATTT	5549
Immutans	CTGCTATGAGTTTT <u>T</u> GAACCTCAGTTTCTTCATATTCTTGTTTTAGA	
Lycopersicon	GCTAGGAGTTTTGAGAAGTCATCAGTTT	
esculentum	AAACTGATGACTTCTCAAAACTCCTAGCTCTAAAACAAGAATATGAA	5550
Gly11Term	GAAACTGAGGTTCAAAAACTCATAGCAGAAATCGAAATCGCCATTC	•
GGA-TGA	TTGATCCTTCCTTCCCACAAGAATC	
	TGAGTTTT <u>T</u> GAACCTCA	5551
·	TGAGGTTC <u>A</u> AAAACTCA	5552
White leaves	GTGGGAAGAAGAAGGATCAAGAATGGCGATTTCGATTTCTGCTA	5553
Immutans	TGAGTTTTGGAACCTGAGTTTCTTCATATTCTTGTTTTAGAGCTAGG	
Lycopersicon	AGTTTTGAGAAGTCATCAGTTTTATGCAA	
esculentum	TTGCATAAAACTGATGACTTCTCAAAACTCCTAGCTCTAAAACAAGA	5554
Ser13Term	ATATGAAGAAACTCAGGTTCCAAAACTCATAGCAGAAATCGAAATC	
TCA-TGA	GCCATTCTTGATCCTTCTTCCTTCCCAC	
	TGGAACCT <u>G</u> AGTTTCTT	5555
	AAGAAACT <u>C</u> AGGTTCCA	5556
White leaves	AAGAAGGATCAAGAATGGCGATTTCGATTTCTGCTATGAGTTTTGG	5557
Immutans	AACCTCAGTTTCTTGATATTCTTGTTTTAGAGCTAGGAGTTTTGAGA	
Lycopersicon	AGTCATCAGTTTTATGCAATTCCCAGAA	
esculentum	TTCTGGGAATTGCATAAAACTGATGACTTCTCAAAACTCCTAGCTC	5558
Ser16Term	TAAAACAAGAATATCAAGAAACTGAGGTTCCAAAACTCATAGCAGA	
TCA-TGA	AATCGAAATCGCCATTCTTGATCCTTCTT	
	AGTITCTT <u>G</u> ATATTCTT	5559
	AAGAATAT <u>C</u> AAGAAACT	5560
White leaves	AGGATCAAGAATGGCGATTTCGATTTCTGCTATGAGTTTTGGAACC	5561
Immutans	TCAGTTTCTTCATAGTCTTGTTTTAGAGCTAGGAGTTTTGAGAAGTC	
Lycopersicon	ATCAGTTTTATGCAATTCCCAGAACCCA	
esculentum	TGGGTTCTGGGAATTGCATAAAACTGATGACTTCTCAAAACTCCTA	5562
Tyr17Term	GCTCTAAAACAAGA <u>C</u> TATGAAGAAACTGAGGTTCCAAAACTCATAG	
TAT-TAG	CAGAAATCGAAATCGCCATTCTTGATCCT	
	TCTTCATA <u>G</u> TCTTGTTT	5563
	AAACAAGACTATGAAGA	5564

	White leaves	AAGAATGGCGATTTCGATTTCTGCTATGAGTTTTGGAACCTCAGTT	5565
	Immutans Lycopersicon	TCTTCATATTCTTGATTTAGAGCTAGGAGTTTTGAGAAGTCATCAGT TTTATGCAATTCCCAGAACCCATGTCGG	
5	esculentum Cys19Term TGT-TGA	CCGACATGGGTTCTGGGAATTGCATAAAACTGATGACTTCTCAAAA CTCCTAGCTCTAAATCAAGAATATGAAGAAACTGAGGTTCCAAAAC TCATAGCAGAAATCGAAATCGCCATTCTT	5566
		TATTCTTG <u>A</u> TTTAGAGC	5567
		GCTCTAAA <u>T</u> CAAGAATA	5568
	White leaves Immutans Capsicum annuum	CGCGTCCGATAAAAAAATCAAGAATGGCGATTTCCATATCTGCTAT GAGTTTTCGAACTTGAGTTTCTTCATATTCAGCATTTTTGTGCA ATTCCAAGAACCCATTTTGTTTGAATTC	5569
10	Ser13Term TCA-TGA	GAATTCAAACAAAATGGGTTCTTGGAATTGCACAAAAATGCTGAAT ATGAAGAAGAAACT <u>C</u> AAGTTCGAAAACTCATAGCAGATATGGAAAT CGCCATTCTTGATTTTTTTATCGGACGCG	5570
		TCGAACTT <u>G</u> AGTTTCTT	5571
		AAGAAACT <u>C</u> AAGTTCGA	5572
	White leaves Immutans Capsicum annuum	AAAAATCAAGAATGGCGATTTCCATATCTGCTATGAGTTTTCGAACT TCAGTTTCTTCGATATTCAGCATTTTTGTGCAATTCCAAGAACCC ATTTTGTTTGAATTCTCTATTTTCACT	5573
15	Ser17Term TCA-TGA	AGTGAAAATAGAGAATTCAAACAAAATGGGTTCTTGGAATTGCACA AAAATGCTGAATAT <u>C</u> AAGAAGAAACTGAAGTTCGAAAACTCATAGC AGATATGGAAATCGCCATTCTTGATTTTT	5574
	·	TTCTTCTT <u>G</u> ATATTCAG	5575
		CTGAATAT <u>C</u> AAGAAGAA	5576
	White leaves Immutans Capsicum annuum	CAAGAATGGCGATTTCCATATCTGCTATGAGTTTTCGAACTTCAGT TTCTTCATATTGAGCATTTTTGTGCAATTCCAAGAACCCATTTT GTTTGAATTCTCTATTTTCACTTAGGAA	5577
20	Ser19Term TCA-TGA	TTCCTAAGTGAAAATAGAGAATTCAAACAAAATGGGTTCTTGGAATT GCACAAAAATGCTCAATATGAAGAAGAAACTGAAGTTCGAAAACTC ATAGCAGATATGGAAATCGCCATTCTTG	5578
		TTCATATT <u>G</u> AGCATTTT	5579
		AAAATGCT <u>C</u> AATATGAA	5580
	White leaves Immutans Capsicum annuum	CGATTTCCATATCTGCTATGAGTTTTCGAACTTCAGTTTCTTCA TATTCAGCATTTTAGTGCAATTCCAAGAACCCATTTTGTTTG	5581
25	Leu21Term	CTATGAGAATTCCTAAGTGAAAATAGAGAATTCAAACAAA	5582
		AGCATTIT <u>A</u> GTGCAATT	5583
		AATTGCAC <u>T</u> AAAATGCT	5584

White leaves	TTCCATATCTGCTATGAGTTTTCGAACTTCAGTTTCTTCATATT	5585
Immutans	CAGCATTTTTGTGAAATTCCAAGAACCCATTTTGTTTGAATTCTCTA	
Capsicum annuum	TTTTCACTTAGGAATTCTCATAGAACT	
Cys22Term	AGTTCTATGAGAATTCCTAAGTGAAAATAGAGAATTCAAACAAA	5586
TGC-TGA	GGTTCTTGGAATT <u>T</u> CACAAAAATGCTGAATATGAAGAAGAAACTGA	
	AGTTCGAAAACTCATAGCAGATATGGAA	
	TTTTTGTG <u>A</u> AATTCCAA	5587
	TTGGAATT <u>T</u> CACAAAAA	5588
White leaves	TTCGGCACGAGGAGAAGGAGCAGACCGAGGTGGCCGTCGAGG	5589
Immutans	AGTCCTTCCCCTTCAGG <u>T</u> AGACGGCTCCTCCTGACGAGCCACTGG	
Oryza sativa	TCACCGCCGAGGAGAGCTGGGTGGTTAAGCTCG	
Glu22Term	CGAGCTTAACCACCCAGCTCTCCTCGGCGGTGACCAGTGGCTCG	5590
GAG-TAG	TCAGGAGGAGCCGTCTACCTGAAGGGGAAGGACTCCTCGACGGC	
	CACCTCGGTCTGCTCCTTCTCCCTCGTGCCGAA	
	CCTTCAGG <u>T</u> AGACGGCT	5591
<u> </u>	AGCCGTCT <u>A</u> CCTGAAGG	5592
White leaves	GAGCAGACCGAGGTGGCCGTCGAGGAGTCCTTCCCCTTCAGGGA	5593
Immutans	GACGGCTCCTCCTGAC <u>T</u> AGCCACTGGTCACCGCCGAGGAGAGCT	
Oryza sativa	GGGTGGTTAAGCTCGAGCAGTCCGTGAACATTT	
Glu28Term	AAATGTTCACGGACTGCTCGAGCTTAACCACCCAGCTCTCCTCGG	5594
CAG-TAG	CGGTGACCAGTGGCT <u>A</u> GTCAGGAGGAGCCGTCTCCCTGAAGGGG	
	AAGGACTCCTCGACGGCCACCTCGGTCTGCTC	
•	CTCCTGAC <u>T</u> AGCCACTG	5595
	CAGTGGCT <u>A</u> GTCAGGAG	5596
White leaves	GTCGAGGAGTCCTTCCCCTTCAGGGAGACGGCTCCTCCTGACGA	5597
Immutans	GCCACTGGTCACCGCCTAGGAGAGCTGGGTGGTTAAGCTCGAGC	
Oryza sativa	AGTCCGTGAACATTTTCCTCACGGAGTCAGTCA	
Glu34Term	TGACTGACTCCGTGAGGAAAATGTTCACGGACTGCTCGAGCTTAA	5598
GAG-TAG	CCACCCAGCTCTCCTAGGCGGTGACCAGTGGCTCGTCAGGAGGA	
	GCCGTCTCCCTGAAGGGAAGGACTCCTCGAC	
	TCACCGCC <u>T</u> AGGAGAGC	5599
	GCTCTCCT <u>A</u> GGCGGTGA	5600
White leaves	GAGGAGTCCTTCCCCTTCAGGGAGACGGCTCCTCCTGACGAGCC	5601
Immutans	ACTGGTCACCGCCGAG <u>T</u> AGAGCTGGGTGGTTAAGCTCGAGCAGT	
Oryza sativa	CCGTGAACATTTTCCTCACGGAGTCAGTCATCA	
Glu35Term	TGATGACTGACTCCGTGAGGAAAATGTTCACGGACTGCTCGAGCT	5602
GAG-TAG	TAACCACCCAGCTCT <u>A</u> CTCGGCGGTGACCAGTGGCTCGTCAGGA	
	GGAGCCGTCTCCCTGAAGGGGAAGGACTCCTC	
	CCGCCGAGTAGAGCTGG	5603
	CCAGCTCTACTCGGCGG	5604

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	White leaves	CTTCCCCTTCAGGGAGACGGCTCCTCCTGACGAGCCACTGGTCACCCGCGAGGAGAGAGCTGAGTGGTTAAGCTCGAGCAGTCCGTGAACA	5605
	Oryza sativa	TTTTCCTCACGGAGTCAGTCATCACGATACTT	
	Trp37Term	AAGTATCGTGATGACTGACTCCGTGAGGAAAATGTTCACGGACTG	5606
5	TGG-TGA	CTCGAGCTTAACCACTCAGCTCTCCTCGGCGGTGACCAGTGGCTC	
		GTCAGGAGGAGCCGTCTCCCTGAAGGGGAAG GAGAGCTGAGTGATAA	5607
		TTAACCACTCAGCTCTC	
	140.2		5608
	White leaves Immutans	TCCGGAGGAGGAGGGGGGATTCGACGAGGAGCTCACCCTCGCCGGCGAGGACGGCGACTGAGTCGTCAGATTCGAGCAGTCCTTCAAC	5609
	Triticum aestivum	GTATTCCTCACGGATACTGTCATCTTTATACTC	
	Trp22Term	GAGTATAAAGATGACAGTATCCGTGAGGAATACGTTGAAGGACTG	5610
10	TGG-TGA	CTCGAATCTGACGAC <u>T</u> CAGTCGCCGTCCTCGCCGGCGAGGGTGA	
		GCTCCTCGTCGAATCCCCCTTCCTCCTCGGA	
		GGCGACTG <u>A</u> GTCGTCAG	5611
		CTGACGACTCAGTCGCC	5612
	White leaves	GAGGAAGGGGATTCGACGAGGAGCTCACCCTCGCCGGCGAGG	5613
	Immutans Triticum aestivum	ACGGCGACTGGGTCGTCTGATTCGAGCAGTCCTTCAACGTATTCC TCACGGATACTGTCATCTTTATACTCGATATTC	•
	Arg25Term	GAATATCGAGTATAAAGATGACAGTATCCGTGAGGAATACGTTGAA	5614
15	AGA-TGA	GGACTGCTCGAATCAGACGACCCAGTCGCCGTCCTCGCCGGCGA	7017
		GGGTGAGCTCCTCGTCGAATCCCCCTTCCTC	
		GGGTCGTC <u>T</u> GATTCGAG	5615
		CTCGAATC <u>A</u> GACGACCC	5616
	White leaves	GGGGGATTCGACGAGGAGCTCACCCTCGCCGGCGAGGACGGCG	5617
	Immutans Triticum aestivum	ACTGGGTCGTCAGATTCTAGCAGTCCTTCAACGTATTCCTCACGGA	
	Glu27Term	TACTGTCATCTTTATACTCGATATTCTGTATC GATACAGAATATCGAGTATAAAGATGACAGTATCCGTGAGGAATAC	5618
20	GAG-TAG	GTTGAAGGACTGCTAGAATCTGACGACCCAGTCGCCGTCCTCGCC	3010
•		GGCGAGGGTGAGCTCCTCGTCGAATCCCCC	
		TCAGATTC <u>T</u> AGCAGTCC	5619
		GGACTGCT <u>A</u> GAATCTGA	5620
	White leaves	GGATTCGACGAGGAGCTCACCCTCGCCGGCGAGGACGGCGACTG	5621
	Immutans Triticum aestivum	GGTCGTCAGATTCGAGTAGTCCTTCAACGTATTCCTCACGGATACT	
	Gin28Term	GTCATCTTTATACTCGATATTCTGTATCGTG CACGATACAGAATATCGAGTATAAAGATGACAGTATCCGTGAGGAA	<b>EC00</b>
25	CAG-TAG	TACGTTGAAGGACTACTCGAATCTGACGACCCAGTCGCCGTCCTC	5622
		GCCGGCGAGGTGAGCTCCTCGTCGAATCC	
		GATTCGAG <u>T</u> AGTCCTTC	5623
		GAAGGACT <u>A</u> CTCGAATC	5624

White leaves Immutans Triticum aestivum	CGAGCAGTCCTTCAACGTATTCCTCACGGATACTGTCATCTTTATA CTCGATATTCTGTAGCGTGACCGCGACTACGCAAGGTTCTTCGTG CTCGAGACCATCGCCAGGGTGCCCTATTTC	5625
Tyr46Term TAT-TAG	GAAATAGGGCACCCTGGCGATGGTCTCGAGCACGAAGAACCTTG CGTAGTCGCGGTCACGCTACAGAATATCGAGTATAAAGATGACAG TATCCGTGAGGAATACGTTGAAGGACTGCTCG	5626
	ATTCTGTA <u>G</u> CGTGACCG	5627
	CGGTCACGCTACAGAAT	5628

## Example 9

## Altering amino acid content of plants

Another aim of biotechnology is to generate plants, especially crop plants, with added value traits. An example of such a trait is improved nutritional quality in food crops. For example, lysine, tryptophan and threonine, which are essential amino acids in the diet of humans and many animals, are limiting nutrients in most cereal crops. Consequently, grain-based diets, such as those based on corn, barley, wheat, rice, maize, millet, sorghum, and the like, must be supplemented with more expensive synthetic amino acids or amino-acid-containing oilseed protein meals. Increasing the lysine content of these grains or of any of the feed component crops would result in significant added value.

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Naturally occurring mutants of plants that have different levels of particular essential amino acids have been identified. However, these mutants are generally not the result of increased free amino acid, but are instead the result of shifts in the overall protein profile of the grain. For example, in maize, reduced levels of lysine-deficient endosperm proteins (prolamines) are complemented by elevated levels of more lysine-rich proteins (albumins, globulins and glutelins). While nutritionally superior, these mutants are associated with reduced yields and poor grain quality, limiting their agronomic usefulness.

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An alternative approach is to generate plants with mutations that render key amino acid biosynthetic enzymes insensitive to feedback inhibition. Many such mutations are known and mutation results in increased free amino acid. The increased production can optionally be coupled to increased expression of an abundant storage protein comprising the chosen amino acid. Alternatively, a normally abundant protein can be engineered to contain more of the target amino acid.

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The attached table discloses exemplary oligonucleotide base sequences which can be used to generate site-specific mutations that remove feedback inhibition in plant amino acid biosynthetic enzymes.

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Table 19
Genome-Altering Oligos Conferring Amino Acid Overproduction

Phenotype, Gene, Plant & Targeted Atteration	Altering Oligos	SEQID NO:
Met Overproduction CGS	TATCCTCCAGGATCTTAAGATTTCCTCCTAATTTCGTCCGTC	5629
<i>Arabidopsis thaliana</i> Arg77His	GATCGTGGCGGCTAAGTGGTCCAACAACCC GGGTTGTTGGACCACTTAGCCGCCACGATCTGTGCAACACCGAT	5630
CGT-CAT	GTTGCTACAGTTTCTATGGGCTTTAATGCTCAGCTGACGGACG	
	TAAAGCCCATAGAAACT	5631
	AGTTTCTA <u>T</u> GGGCTTTA	5632
Met Overproduction CGS Arabidopsis thaliana	TCTTAAGATTTCCTCCTAATTTCGTCCGTCAGCTGAGCATTAAAGC CCGTAGAAACTGTAACAACATCGGTGTTGCACAGATCGTGGCGG CTAAGTGGTCCAACAACCCATCCTCCGCGTT	5633
Ser81Asn AGC-AAC	AACGCGGAGGATGGGTTGTTGGACCACTTAGCCGCCACGATCTG TGCAACACCGATGTTGTTACAGTTTCTACGGGCTTTAATGCTCAGC TGACGGACGAAATTAGGAGGAAATCTTAAGA	5634
	AAACTGTA <u>A</u> CAACATCG	5635
	CGATGTTG <u>T</u> TACAGTTT	5636
Met Overproduction CGS Arabidopsis thaliana	TTTCCTCCTAATTTCGTCCGTCAGCTGAGCATTAAAGCCCGTAGAA ACTGTAGCAACATCAGTGTTGCACAGATCGTGGCGGCTAAGTGGT CCAACAACCCATCCTCCGCGTTACCTTCGG	5637
Gly84Ser GGT-AGT	CCGAAGGTAACGCGGAGGATGGGTTGTTGGACCACTTAGCCGCC ACGATCTGTGCAACACTGATGTTGCTACAGTTTCTACGGGCTTTAA TGCTCAGCTGACGGACGAAATTAGGAGGAAA	5638
	GCAACATC <u>A</u> GTGTTGCA	5639
	TGCAACAC <u>T</u> GATGTTGC	5640
Met Overproduction CGS Arabidopsis thaliana	TTCCTCCTAATTTCGTCCGTCAGCTGAGCATTAAAGCCCGTAGAAA CTGTAGCAACATCGATGTTGCACAGATCGTGGCGGCTAAGTGGTC CAACAACCCATCCTCCGCGTTACCTTCGGC	5641
Gly84Asp GGT-GAT	GCCGAAGGTAACGCGGAGGATGGGTTGTTGGACCACTTAGCCGC CACGATCTGTGCAACATCGATGTTGCTACAGTTTCTACGGGCTTTA ATGCTCAGCTGACGGACGAAATTAGGAGGAA	5642
	CAACATCGATGTTGCAC	5643
	GTGCAACA <u>T</u> CGATGTTG	5644

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Met Overproduction CGS Fragraria vesca	TATCGTCACTCATCCTCCGCTTCCCTCCCAACTTCGTCCGCCAGC TCAGCACCAAGGCCCACCGCAACTGCAGCAACATCGGCGTCGCG CAGATCGTCGCGGCTTCGTGGTCCAACAAAGA	5645
Arg73His CGC-CAC	TCTTTGTTGGACCACGAAGCCGCGACGATCTGCGCGACGCCGATGTTGCTGCAGTTGCGGTGGGCCTTGGTGCTGAGCTGGCGGACGACGAAGTTGGGAGGAAGCGGAGGATGAGTGACGATA	5646
	CAAGGCCC <u>A</u> CCGCAACT	5647
	AGTTGCGGTGGGCCTTG	5648
Met Overproduction CGS Fragraria vesca	TCCTCCGCTTCCCTCCCAACTTCGTCCGCCAGCTCAGCACCAAG GCCCGCCGCAACTGCAACAACATCGGCGTCGCGCAGATCGTCGC GGCTTCGTGGTCCAACAAAGACTCCGACCTTTC	5649
Ser77Asn AGC-AAC	GAAAGGTCGGAGTCTTTGTTGGACCACGAAGCCGCGACGATCTG CGCGACGCCGATGTTGTTGCAGTTGCGGCGGGCCTTGGTGCTGA GCTGGCGGACGAAGTTGGGAGGGAAGCGGAGGA	5650
	CAACTGCA <u>A</u> CAACATCG	5651
	CGATGTTGTTGCAGTTG	5652
Met Overproduction CGS Fragraria vesca	TTCCTCCCAACTTCGTCCGCCAGCTCAGCACCAAGGCCCGCCG CAACTGCAGCAACATCAGCGTCGCGCAGATCGTCGCGGCTTCGT GGTCCAACAAAGACTCCGACCTTTCGGCGGTGC	5653
Gly80Ser GGC-AGC	GCACCGCCGAAAGGTCGGAGTCTTTGTTGGACCACGAAGCCGCG ACGATCTGCGCGACGC <u>I</u> GATGTTGCTGCAGTTGCGGCGGGCCTT GGTGCTGAGCTGGCGGACGAAGTTGGGAGGGAA	5654
	GCAACATC <u>A</u> GCGTCGCG	5655
	CGCGACGC <u>T</u> GATGTTGC	5656
Met Overproduction CGS Fragraria vesca	TCCCTCCCAACTTCGTCCGCCAGCTCAGCACCAAGGCCCGCCGCAACTGCAGCAACATCGACGTCGCGCAGATCGTCGCGGCTTCGTGGTCCAACAAAGACTCCGACCTTTCGGCGGTGCC	5657
Gly80Asp GGC-GAC	GGCACCGCGAAAGGTCGGAGTCTTTGTTGGACCACGAAGCCGC GACGATCTGCGCGACGTCGATGTTGCTGCAGTTGCGGCGGGCCT TGGTGCTGAGCTGGCGGACGAAGTTGGGAGGGA	5658
	CAACATCG <u>A</u> CGTCGCGC	5659
	GCGCGACG <u>T</u> CGATGTTG	5660
Met Overproduction CGS Glycine max	TCTCCTCCTCATCCTCCGCTTCCCTCCCAACTTCCAGCGCCAGC TAAGCACCAAGGCGAGCCGCGCAACTGCAGCAACATCGGCGTCGCG CAAATCGTCGCCGCTTCGTGGTCGAACAACAG	5661
Arg68His CGC-CAC	CTGTTGTTCGACCACGAAGCGGCGACGACGACGCCGAT GTTGCTGCAGTTGCGGCTCGCCTTGGTGCTTAGCTGGCGCTGGA AGTTGGGAGGGAAGCGGAGGATGAGGGAGAGA	5662
	CCAAGGCGAGCCGCAAC	5663

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
	GTTGCGGC <u>T</u> CGCCTTGG	5664
Met Overproduction CGS Glycine max	TCCTCCGCTTCCCTCCCAACTTCCAGCGCCAGCTAAGCACCAAG GCGCGCCGCAACTGCAACAACATCGGCGTCGCGCAAATCGTCGC CGCTTCGTGGTCGAACAACAGCGACAACTCTCC	5665
Ser72Asn AGC-AAC	GGAGAGTTGTCGCTGTTGTTCGACCACGAAGCGGCGACGATTTG CGCGACGCCGATGTTGTGCAGTTGCGGCGCGCCTTGGTGCTTA GCTGGCGCTGGAAGTTGGGAGGGAAGCGGAGGA	5666
	CAACTGCAACATCG	5667
	CGATGTTG <u>T</u> TGCAGTTG	5668
Met Overproduction CGS Glycine max	TTCCCTCCCAACTTCCAGCGCCAGCTAAGCACCAAGGCGCGCCG CAACTGCAGCAACATCAGCGTCGCGCAAATCGTCGCCGCTTCGT GGTCGAACAACAGCGACAACTCTCCGGCCGCCG	5669
Gly75Ser GGC-AGC	CGGCGGCCGAGAGTTGTCGCTGTTGTTCGACCACGAAGCGGC GACGATTTGCGCGACGCTGATGTTGCTGCAGTTGCGGCGCGCCCT TGGTGCTTAGCTGGCGCTGGAAGTTGGGAGGGAA	5670
	GCAACATC <u>A</u> GCGTCGCG	5671
	CGCGACGC <u>T</u> GATGTTGC	5672
Met Overproduction CGS Glycine max	TCCCTCCCAACTTCCAGCGCCAGCTAAGCACCAAGGCGCGCCGC AACTGCAGCAACATCGACGTCGCGCAAATCGTCGCCGCTTCGTG GTCGAACAACAGCGACAACTCTCCGGCCGCCGG	5673
Gly75Asp GGC-GAC	CCGGCGGCCGAGAGTTGTCGCTGTTGTTCGACCACGAAGCGGC GACGATTTGCGCGACGTCGATGTTGCTGCAGTTGCGGCGCGCCT TGGTGCTTAGCTGGCGCTGGAAGTTGGGAGGGA	5674
	CAACATCGACGTCGCGC	5675
	GCGCGACG <u>T</u> CGATGTTG	5676
Met Overproduction CGS Solanum tuberosum	TGTCTTCTCGATTTTCAGGTTTCCTCCTAATTTCGTGAGGCAGCT AAGCATTAAGGCT <u>CAC</u> AGGAATTGCAGCAATATTGGCGTGGCTCA AGTTGTGGCGGCTTCCTGGTCTAACAACCA	5677
Arg70His AGG-CAC	TGGTTGTTAGACCAGGAAGCCGCCACAACTTGAGCCACGCCAATA TTGCTGCAATTCCTGTGAGCCTTAATGCTTAGCTGCCTCACGAAAT TAGGAGGAAACCTGAAAATCAGAGAAGACA	5678
	TAAGGCT <u>CAC</u> AGGAATT	5679
	AATTCCT <u>GTG</u> AGCCTTA	5680
Met Overproduction CGS Solanum tuberosum	TTTTCAGGTTTCCTCCTAATTTCGTGAGGCAGCTAAGCATTAAGGC TAGGAGGAATTGCAACAATATTGGCGTGGCTCAAGTTGTGGCGG CTTCCTGGTCTAACAACCAAGCCGGTCCTGA	5681
Ser74Asn AGC-AAC	TCAGGACCGCCTTGGTTGTTAGACCAGGAAGCCGCCACAACTTG AGCCACGCCAATATTGTTGCAATTCCTCCTAGCCTTAATGCTTAGC TGCCTCACGAAATTAGGAGGAAACCTGAAAA	5682

Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID NO:
	GAATTGCA <u>A</u> CAATATTG	5683
	CAATATTG <u>T</u> TGCAATTC	5684
Met Overproduction CGS Solanum tuberosum	TTTCCTCCTAATTTCGTGAGGCAGCTAAGCATTAAGGCTAGGAGG AATTGCAGCAATATTAGCGTGGCTCAAGTTGTGGCGGCTTCCTGG TCTAACAACCAAGCCGGTCCTGAATTCACTC	5685
Gly77Ser GGC-AGC	GAGTGAATTCAGGACCGGCTTGGTTGTTAGACCAGGAAGCCGCC ACAACTTGAGCCACGCTAATATTGCTGCAATTCCTCCTAGCCTTAA TGCTTAGCTGCCTCACGAAATTAGGAGGAAA	5686
	GCAATATT <u>A</u> GCGTGGCT	5687
	AGCCACGC <u>T</u> AATATTGC	5688
Met Overproduction CGS Solanum tuberosum	TTCCTCCTAATTTCGTGAGGCAGCTAAGCATTAAGGCTAGGAGGA ATTGCAGCAATATTGACGTGGCTCAAGTTGTGGCGGCTTCCTGGT CTAACAACCAAGCCGGTCCTGAATTCACTCC	5689
Gly77Asp GGC-GAC	GGAGTGAATTCAGGACCGGCTTGGTTGTTAGACCAGGAAGCCGC CACAACTTGAGCCACGTCAATATTGCTGCAATTCCTCCTAGCCTTA ATGCTTAGCTGCCTCACGAAATTAGGAGGAA	5690
	CAATATTG <u>A</u> CGTGGCTC	5691
	GAGCCACG <u>T</u> CAATATTG	5692
Met Overproduction CGS Mesembryanthemum	CTTCCTCTTATCCTTCGCTTTCCTCCCAACTTTGTCCGTCAGCT CAGCACCAAGGCTCGCCACAACTGCAGCAACATTGGTGTCGCAC AGGTCGTCGCTGCCTCCTGGTCCAACAACTC	5693
<i>crystallinum</i> Arg73His CGC-CAC	GAGTTGTTGGACCAGGAGGCAGCGACGACCTGTGCGACACCAAT GTTGCTGCAGTTG <u>T</u> GGCGAGCCTTGGTGCTGAGCTGACGGACAA AGTTGGGAGGAAAGCGAAGGATAAGAGAGGAAG	5694
	GGCTCGCC <u>A</u> CAACTGCA	5695
	TGCAGTTG <u>T</u> GGCGAGCC	5696
Met Overproduction CGS Mesembryanthemum	TCCTTCGCTTTCCTCCCAACTTTGTCCGTCAGCTCAGCACCAAGG CTCGCCGCAACTGCAACAACATTGGTGTCGCACAGGTCGTCGCT GCCTCCTGGTCCAACAACTCCGATGCCGGCGC	5697
crystallinum Ser77Asn AGC-AAC	GCGCCGGCATCGGAGTTGTTGGACCAGGAGGCAGCGACCCT GTGCGACACCAATGTTGTTGCAGTTGCGGCGAGCCTTGGTGCTG AGCTGACGGACAAAGTTGGGAGGAAAGCGAAGGA	5698
	CAACTGCA <u>A</u> CAACATTG	5699
	CAATGTTGTTGCAGTTG	5700

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Met Overproduction CGS Mesembryanthemum	TTTCCTCCCAACTTTGTCCGTCAGCTCAGCACCAAGGCTCGCCGC AACTGCAGCAACATTAGTGTCGCACAGGTCGTCGCTGCCTCCTG GTCCAACAACTCCGATGCCGGCGCCACCTCTT	5701
crystallinum Gly80Ser GGT-AGT	AAGAGGTĞGCĞCĞĞCĂTCGĞAGTTGTTGGACCAGGAGGCAGC GACGACCTGTGCGACAC <u>T</u> AATGTTGCTGCAGTTGCGGCGAGCCT TGGTGCTGAGCTGA	5702
	GCAACATT <u>A</u> GTGTCGCA	5703 5704
Met Overproduction	TGCGACACTAATGTTGC TTCCTCCCAACTTTGTCCGTCAGCTCAGC	5704
CGS Mesembryanthemum	ACTGCAGCAACATTGATGTCGCACAGGTCGTCGCTCCTGG TCCAACAACTCCGATGCCGGCGCCACCTCTTG	
crystallinum Gly80Asp GGT-GAT	CAAGAGGTGGCGCCGGCATCGGAGTTGTTGGACCAGGAGGCAG CGACGACCTGTGCGACA <u>T</u> CAATGTTGCTGCAGTTGCGGCGAGCC TTGGTGCTGAGCTGA	5706
·	CAACATTGATGTCGCAC GTGCGACATCAATGTTG	5707 5708
Met Overproduction CGS Zea mays	CCTCTGCTACCATCTCGCCTTTCCGCCAAACTTTGTCCGCCAGC TTAGCACCAAGGCACACGCGCAACTGCAGCAACATCGGCGTCGCG CAGATCGTCGCCGCCGCGTGGTCCGACTGCCC	5709
Arg41His CGC-CAC	GGGCAGTCGGACCACGCGGCGGCGACGATCTGCGCGACGCCGA TGTTGCTGCAGTTGCGGTGTGCCTTGGTGCTAAGCTGGCGGACA AAGTTTGGCGGAAAGCGGAGGATGGTAGCAGAGG	5710
	CAAGGCACACCGCAACT	5711
Met Overproduction CGS Zea mays	AGTTGCGGTGTGCCTTG  TCCTCCGCTTTCCGCCAAACTTTGTCCGCCAGCTTAGCACCAAGG CACGCCGCAACTGCAACATCGGCGTCGCGCAGATCGTCGCC GCCGCGTGGTCCGACTGCCCCGCCGCCGCCCCCC	5712 5713
Ser45Asn AGC-AAC	GGGCGAGCGGGGGGCAGTCGGACCACGCGGCGCGACGATC TGCGCGACGCCGATGTTGTTGCAGTTGCGGCGTGCCTTGGTGCT AAGCTGGCGGACAAAGTTTGGCGGAAAGCGGAGGA	5714
	CAACTGCAACATCG	5715
M-10	CGATGTTGTTGCAGTTG	5716
Met Overproduction CGS Zea mays	TTTCCGCCAAACTTTGTCCGCCAGCTTAGCACCAAGGCACGCCGCAACTGCAGCAACATCAGCGTCGCGCAGATCGTCGCCGCCGCGTGGTCCGACTTAG	5717
Gly48Ser GGC-AGC	CTAAGTGGGGGCGAGCGGGGGGGGGCAGTCGGACCACGCGGCGG CGACGATCTGCGCGACGCTGATGTTGCTGCAGTTGCGGCGTGCC TTGGTGCTAAGCTGGCGGACAAAGTTTGGCGGAAA	5718
	GCAACATCAGCGTCGCG	5719

Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID NO:
	CGCGACGCTGATGTTGC	5720
Met Overproduction CGS Zea mays	TTCCGCCAAACTTTGTCCGCCAGCTTAGCACCAAGGCACGCCGCAACTGCAGCAACATCGACGTCGCGCAGATCGTCGCCGCGCGCG	5721
Gly48Asp GGC-GAC	CCTAAGTGGGGCGAGCGGCGGGGGCAGTCGGACCACGCGGCGGCGGCGACGACCGAC	5722
	CAACATCGACGTCGCGC GCGCGACGTCGATGTTG	5723
Met Overproduction	GTATGAATGATCTGTGGGTGAAACACTGTGGGATTAGTCATACAG	5724
TS Arabidopsis thaliana	GAAGTTTCAAGGATCGTGGAATGACTGTTTTGGTTAGTCAAGTTAA TCGTCTGAGAAAGATGAAACGACCTGTGGT	5725
Leu205Arg CTT-CGT	ACCACAGGTCGTTTCATCTTTCTCAGACGATTAACTTGACTAACCA AAACAGTCATTCCACGATCCTTGAAACTTCCTGTATGACTAATCCC ACAGTGTTTCACCCACAGATCATTCATAC	5726
	CAAGGATC <u>G</u> TGGAATGA	5727
	TCATTCCACGATCCTTG	5728
Met Overproduction TS Solanum tuberosum	GCATGACTGATTTGTGGGTCAAACACTGTGGGATTAGCCATACTG GTAGTTTTAAGGATCGTGGGATGACTGTTTTGGTGAGTCAAGTTAA TCGCTTGCGGAAAATGCATAAACCGGTTGT	5729
Leu198Arg CTT-CGT	ACAACCGGTTTATGCATTTTCCGCAAGCGATTAACTTGACTCACCA AAACAGTCATCCCACGATCCTTAAAACTACCAGTATGGCTAATCCC ACAGTGTTTGACCCACAAATCAGTCATGC	5730
	TAAGGATC <u>G</u> TGGGATGA	5731
	TCATCCCA <u>C</u> GATCCTTA	5732
Lys Overproduction DHPS Zea mays	TCATTGGGCACACAGTGAACTGCTTTGGCTCTAGAATCAAAGTGA TAGGCAACACAGGAAACACCAACCAGAGAAGCCGTCCACGCA ACAGAACAGGGATTTGCTGTTGGCATGCATGC	5733
Ser157Asn AGC-AAC	GCATGCATGCCAACAGCAAATCCCTGTTCTGTTGCGTGGACGGCT TCTCTGGTTGAGTTGTTCCTGTGTTGCCTATCACTTTGATTCTAG AGCCAAAGCAGTTCACTGTGTGCCCAATGA	5734
	CACAGGAA <u>A</u> CAACTCAA	5735
	TTGAGTTGTTTCCTGTG	5736
Lys Overproduction DHPS Zea mays	GCTCTAGAATCAAAGTGATAGGCAACACAGGAAGCAACTCAACCA GAGAAGCCGTCCACGAAACAGAACAG	<b>5737</b>
Ala166Val GCA-GAA	CCGTAGTAAGGATTGATGTGGAGAGCCGCATGCATGCCAACAGC AAATCCCTGTTCTGTT	5738

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	CGTCCACG <u>A</u> AACAGAAC	5739
	GTTCTGTT <u>T</u> CGTGGACG	5740
Lys Overproduction	GGCTCTAGAATCAAAGTGATAGGCAACACAGGAAGCAACTCAACC	5741
DHPS	AGAGAAGCCGTCCAC <u>A</u> CAACAGAACAGGGATTTGCTGTTGGCAT	
Zea mays	GCATGCGGCTCTCCACATCAATCCTTACTACG	
Ala166Thr	CGTAGTAAGGATTGATGTGGAGAGCCGCATGCATGCCAACAGCAA	5742
GCA-ACA	ATCCCTGTTCTGTTGTGGACGGCTTCTCTGGTTGAGTTGCTTC CTGTGTTGCCTATCACTTTGATTCTAGAGCC	
	CCGTCCACACAACAGAA	5743
	TTCTGTTGTGGACGG	5744
Luc Ouerreduction	TTATTGGGCATACAGTTAACTGCTTTGGCACTAAAATTAAAGTGGT	5745
Lys Overproduction DHRS	CGGCAACACAGGAAATAACTCAACAAGGGAGGCTATTCACGCAAC	5/45
Oryza sativa	TGAGCAGGGATTCGCTGTAGGTATGCACGC	
Ser124Asn	GCGTGCATACCTACAGCGAATCCCTGCTCAGTTGCGTGAATAGCC	5746
AGT-AAT	TCCCTTGTTGAGTTATTTCCTGTGTTGCCGACCACTTTAATTTTAGT	
	GCCAAAGCAGTTAACTGTATGCCCAATAA	
	CACAGGAA <u>A</u> TAACTCAA	5747
	TTGAGTTATTCCTGTG	5748
Lys Overproduction	GCACTAAAATTAAAGTGGTCGGCAACACAGGAAGTAACTCAACAA	5749
DHPS	GGGAGGCTATTCACGTAACTGAGCAGGGATTCGCTGTAGGTATG	
Oryza sativa	CACGCGGCTCTCCACATCATCCTTACTACGG	F750
Ala133Val GCA-GTA	CCGTAGTAAGGATTGATGTGGAGAGCCGCGTGCATACCTACAGC GAATCCCTGCTCAGTTACGTGAATAGCCTCCCTTGTTGAGTTACTT	5750
GOA-GIA	CCTGTGTTGCCGACCACTTTAATTTTAGTGC	
	TATTCACGTAACTGAGC	5751
	GCTCAGTTACGTGAATA	5752
Lys Overproduction	I GGCACTAAAATTAAAGTGGTCGGCAACACAGGAAGTAACTCAACA	5753
DHPS	AGGGAGGCTATTCACACAACTGAGCAGGGATTCGCTGTAGGTAT	
Oryza sativa	GCACGCGCTCTCCACATCATCCTTACTACG	
Ala133Thr	CGTAGTAAGGATTGATGTGGAGAGCCGCGTGCATACCTACAGCG	5754
GCA-ACA	AATCCCTGCTCAGTTGTGTGAATAGCCTCCCTTGTTGAGTTACTTC	
	CTGTGTTGCCGACCACTTTAATTTTAGTGCC CTATTCACACAACTGAG	5755
1	CTCAGTTGTGTGAATAG	5756
Lys Overproduction DHPS 1	TCATCGGGCATACTGTTAACTGCTTTGGAGCCAACATTAAAGTGAT AGGCAACACGGGAAATAACTCAACCAGAGAAGCTGTTCACGCGA	5757
Triticum aestivum	CAGAGCAGGGATTTGCTGTTGGCATGCAGGAGGAGCTGTTCACGCGA	
Ser165Asn		

Ser165Asn AGT-AAT

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	GCATGCATGCCAACAGCAAATCCCTGCTCTGTCGCGTGAACAGCT TCTCTGGTTGAGTTA_TTCCCGTGTTGCCTATCACTTTAATGTTGG CTCCAAAGCAGTTAACAGTATGCCCGATGA	5758
	CACGGGAA <u>A</u> TAACTCAA TTGAGTTA <b>T</b> TTCCCGTG	5759 5760
Lys Overproduction DHPS 1 Triticum aestivum	GAGCCAACATTAAAGTGATAGGCAACACGGGAAGTAACTCAACCA GAGAAGCTGTTCACGTGACAGAGCAGGGATTTGCTGTTGGCATG CATGCAGCTCTTCATGTCAATCCTTACTACGG	5761
Ala174Val GCG-GTG	CCGTAGTAAGGATTGACATGAAGAGCTGCATGCCAACAGCA AATCCCTGCTCTGCACGTGAACAGCTTCTCTGGTTGAGTTACTT CCCGTGTTGCCTATCACTTTAATGTTGGCTC	5762
	TGTTCACGTGACAGAGC	5763
Lys Overproduction	GCTCTGTCACGTGAACA  GGAGCCAACATTAAAGTGATAGGCAACACGGGAAGTAACTCAACC	5764 5765
DHPS 1 Triticum aestivum	AGAGAAGCTGTTCACACGACAGAGCAGGGATTTGCTGTTGGCAT GCATGCAGCTCTTCATGTCAATCCTTACTACG	
Ala174Thr GCG-ACG	CGTAGTAAGGATTGACATGAAGAGCTGCATGCATGCCAACAGCAA ATCCCTGCTCTGTCGTGTGAACAGCTTCTCTGGTTGAGTTACTTC CCGTGTTGCCTATCACTTTAATGTTGGCTCC	5766
	CTGTTCAC <u>A</u> CGACAGAG	5767
Luc Ouerne dustin	CTCTGTCGTGTGAACAG	5768
Lys Overproduction DHPS 2 Triticum aestivum	TCATCGGGCACACTGTTAACTGCTTTGGAACTAACATTAAAGTGAT AGGCAACACGGGAAATAACTCAACTAGAGAAGCGATTCACGCTTC AGAGCAGGGATTTGCTGTTGGCATGCATGC	5769
Ser154Asn AGT-AAT	GCATGCATGCCAACAGCAAATCCCTGCTCTGAAGCGTGAATCGCT TCTCTAGTTGAGTTATTTCCCGTGTTGCCTATCACTTTAATGTTAGT TCCAAAGCAGTTAACAGTGTGCCCGATGA	5770
	CACGGGAA <u>A</u> TAACTCAA	5771
	TTGAGTTATTTCCCGTG	5772
Lys Overproduction DHPS 2 Triticum aestivum	GAACTAACATTAAAGTGATAGGCAACACGGGAAGTAACTCAACTA GAGAAGCGATTCACGTTTCAGAGCAGGGATTTGCTGTTGGCATGC ATGCAGCTCTCCATGTCAATCCTTACTATGG	5773
Ala163Val GCT-GTT	CCATAGTAAGGATTGACATGGAGAGCTGCATGCCAACAGCA AATCCCTGCTCTGAAACGTGAATCGCTTCTCTAGTTGAGTTACTTC CCGTGTTGCCTATCACTTTAATGTTAGTTC	5774
	GATTCACG <u>T</u> TTCAGAGC	5775
	GCTCTGAAACGTGAATC	5776

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Lys Overproduction DHPS 2 Triticum aestivum	GGAACTAACATTAAAGTGATAGGCAACACGGGAAGTAACTCAACT AGAGAAGCGATTCACACTTCAGAGCAGGGATTTGCTGTTGGCATG CATGCAGCTCTCCATGTCAATCCTTACTATG	5777
Ala163Thr GCT-ACT	CATAGTAAGGATTGACATGGAGAGCTGCATGCATGCCAACAGCAA ATCCCTGCTCTGAAG <u>T</u> GTGAATCGCTTCTCTAGTTGAGTTACTTCC CGTGTTGCCTATCACTTTAATGTTAGTTCC	5778
	CGATTCAC <u>A</u> CTTCAGAG	5779
	CTCTGAAG <u>T</u> GTGAATCG	5780
Lys Overproduction DHPS Coix lacryma-jobi	CTCATTGGGCATACTGTGAACTGCTTTGGCTCTAGAATTAAAGTGA TAGGCAACACAGGAAATAACTCAACCAGAGAAGCTGTTCACGCAA CAGAGCAGGGATTTGCTGTTGGCATGCATG	5781
Ser154Asn AGT-AAT	CATGCATGCCAACAGCAAATCCCTGCTCTGTTGCGTGAACAGCTT CTCTGGTTGAGTTATTTCCTGTGTTGCCTATCACTTTAATTCTAGA GCCAAAGCAGTTCACAGTATGCCCAATGAG	5782
	CACAGGAAATAACTCAA	5783
	TTGAGTTA <u>T</u> TTCCTGTG	5784
Lys Overproduction DHPS Coix lacryma-jobi	GCTCTAGAATTAAAGTGATAGGCAACACAGGAAGTAACTCAACCA GAGAAGCTGTTCACGTAACAGAGCAGGGATTTGCTGTTGGCATGC ATGCAGCTCTCCACATCAATCCTTACTATGG	5785
Ala163Val GCA-GTA	CCATAGTAAGGATTGATGTGGAGAGCTGCATGCCAACAGCA AATCCCTGCTCTGTTACGTGAACAGCTTCTCTGGTTGAGTTACTTC CTGTGTTGCCTATCACTTTAATTCTAGAGC	5786
	TGTTCACG <u>T</u> AACAGAGC	5787
	GCTCTGTT <u>A</u> CGTGAACA	5788
Lys Overproduction DHPS Coix lacryma-jobi	GGCTCTAGAATTAAAGTGATAGGCAACACAGGAAGTAACTCAACC AGAGAAGCTGTTCACAACAGAGCAGGGATTTGCTGTTGGCATG CATGCAGCTCTCCACATCAATCCTTACTATG	5789
Ala163Thr GCA-ACA	CATAGTAAGGATTGATGTGGAGAGCTGCATGCATGCCAACAGCAA ATCCCTGCTCTGTTGTGTGAACAGCTTCTCTGGTTGAGTTACTTCC TGTGTTGCCTATCACTTTAATTCTAGAGCC	5790
	CTGTTCAC <u>A</u> CAACAGAG	5791
	CTCTGTTG <u>T</u> GTGAACAG	5792
Lys Overproduction DHPS Nicotiana tabacum	TCATTGGTCACACAGTCAATTGTTTTGGAGGGTCCATCAAAGTCAT CGGGAACACTGGAAACAACTCCACAAGGGAAGCAATCCATGCAA CTGAACAGGGATTTGCTGTAGGTATGCATGC	5793
Ser136Asn AGC-AAC	GCATGCATACCTACAGCAAATCCCTGTTCAGTTGCATGGATTGCTT CCCTTGTGGAGTTGTTCCAGTGTTCCCGATGACTTTGATGGACC CTCCAAAACAATTGACTGTGTGACCAATGA	5794
	CACTGGAA <u>A</u> CAACTCCA	5795

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	TGGAGTTGTTTCCAGTG	5796
Lys Overproduction DHPS Nicotiana tabacum	GAGGGTCCATCAAAGTCATCGGGAACACTGGAAGCAACTCCACAA GGGAAGCAATCCATGTAACTGAACAGGGATTTGCTGTAGGTATGC ATGCAGCTCTTCACATTAATCCCTACTATGG	5797
Ala145Val GCA-GTA	CCATAGTAGGGATTAATGTGAAGAGCTGCATGCATACCTACAGCA AATCCCTGTTCAGTTACATGGATTGCTTCCCTTGTGGAGTTGCTTC CAGTGTTCCCGATGACTTTGATGGACCCTC	5798
	AATCCATG <u>T</u> AACTGAAC	5799
	GTTCAGTT <u>A</u> CATGGATT	5800
Lys Overproduction DHPS Nicotiana tabacum	GGAGGGTCCATCAAAGTCATCGGGAACACTGGAAGCAACTCCAC AAGGGAAGCAATCCATACAACTGAACAGGGATTTGCTGTAGGTAT GCATGCAGCTCTTCACATTAATCCCTACTATG	5801
Ala145Thr GCA-ACA	CATAGTAGGGATTAATGTGAAGAGCTGCATGCATACCTACAGCAA ATCCCTGTTCAGTTGTATGGATTGCTTCCCTTGTGGAGTTGCTTCC AGTGTTCCCGATGACTTTGATGGACCCTCC	5802
· ·	CAATCCAT <u>A</u> CAACTGAA	5803
	TTCAGTTG <u>T</u> ATGGATTG	5804
Lys Overproduction DHPS Arabidopsis thaliana	TTATAGGCCATACCGTTAACTGTTTTGGCGGAAGCATCAAAGTCAT TGGAAACACTGGAAACAATTCGACTAGAGAAGCAATCCACGCGAC TGAACAAGGATTCGCGGTTGGAATGCATGC	5805
Ser142Asn AGC-AAC	GCATGCATTCCAACCGCGAATCCTTGTTCAGTCGCGTGGATTGCT TCTCTAGTCGAATTGTTTCCAGTGTTTCCAATGACTTTGATGCTTC CGCCAAAACAGTTAACGGTATGGCCTATAA	5806
	CACTGGAA <u>A</u> CAATTCGA	5807
	TCGAATTG <u>T</u> TTCCAGTG	5808
Lys Overproduction DHPS Arabidopsis thaliana	GCGGAAGCATCAAAGTCATTGGAAACACTGGAAGCAATTCGACTA GAGAAGCAATCCACGTGACTGAACAAGGATTCGCGGTTGGAATGC ATGCTGCTCTTCATATAAACCCTTACTATGG	5809
Ala151Val GCG-GTG	CCATAGTAAGGGTTTATATGAAGAGCAGCATGCATTCCAACCGCG AATCCTTGTTCAGTCACGTGGATTGCTTCTCTAGTCGAATTGCTTC CAGTGTTTCCAATGACTTTGATGCTTCCGC	5810
	AATCCACG <u>T</u> GACTGAAC	5811
	GTTCAGTC <u>A</u> CGTGGATT	5812
Lys Overproduction DHPS Arabidopsis thaliana	GGCGGAAGCATCAAAGTCATTGGAAACACTGGAAGCAATTCGACT AGAGAAGCAATCCACACGACTGAACAAGGATTCGCGGTTGGAATG CATGCTGCTCTTCATATAAACCCTTACTATG	5813
Ala151Thr GCG-ACG	CATAGTAAGGGTTTATATGAAGAGCAGCATGCATTCCAACCGCGA ATCCTTGTTCAGTCGTGTGGATTGCTTCTCTAGTCGAATTGCTTCC AGTGTTTCCAATGACTTTGATGCTTCCGCC	5814

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	CAATCCAC <u>A</u> CGACTGAA	5815
	TTCAGTCGTGTGGATTG	5816
Lys Overproduction	TTATTGCTCATACAGTCAACTGTTTTGGTGGGAAAATTAAGGTTATT	5817
DHPS	GGAAATACTGGAA <u>A</u> CAACTCCACCAGGGAAGCAATTCATGCCACT	
Glycine max	GAGCAGGGTTTTGCTGTTGGAATGCATGC	-212
Ser103Asn	GCATGCATTCCAACAGCAAAACCCTGCTCAGTGGCATGAATTGCT	5818
AGC-AAC	TCCCTGGTGGAGTTGTTTCCAGTATTTCCAATAACCTTAATTTTCC	
	CACCAAAACAGTTGACTGTATGAGCAATAA TACTGGAAACAACTCCA	5819
	TGGAGTTGTTTCCAGTA	5820
Lys Overproduction	GTGGGAAAATTAAGGTTATTGGAAATACTGGAAGCAACTCCACCA	5821
DHPS	GGGAAGCAATTCATGTCACTGAGCAGGGTTTTGCTGTTGGAATGC	
Glycine max Ala112Val	ATGCTGCCCTTCACATAAACCCTTACTATGG CCATAGTAAGGGTTTATGTGAAGGGCAGCATGCATTCCAACAGCA	5822
GCC-GTC	AAACCCTGCTCAGTGACATGAATTGCTTCCCTGGTGGAGTTGCTT	3022
,	CCAGTATTTCCAATAACCTTAATTTTCCCAC	
	AATTCATGTCACTGAGC	5823
	GCTCAGTGACATGAATT	5824
Lys Overproduction	I GGTGGGAAAATTAAGGTTATTGGAAATACTGGAAGCAACTCCACC	5825
DHPS	AGGGAAGCAATTCATACCACTGAGCAGGGTTTTGCTGTTGGAATG	
Glycine max	CATGCTGCCCTTCACATAAACCCTTACTATG	
Ala112Thr	CATAGTAAGGGTTTATGTGAAGGGCAGCATGCATTCCAACAGCAA	5826
GCC-ACC	AACCCTGCTCAGTGG <u>T</u> ATGAATTGCTTCCCTGGTGGAGTTGCTTC	
	CAGTATTTCCAATAACCTTAATTTTCCCACC	
	CAATTCAT <u>A</u> CCACTGAG	5827
	CTCAGTGG <u>T</u> ATGAATTG	5828
Trp Overproduction	CTTGCAGGAGACATATTTCAGATCGTGCTGAGTCAACGTTTTGAG	5829
AS	CGGCGAACATTTGCAAACCCCTTTGAAGTTTATAGAGCACTAAGA	
Arabidopsis thaliana	GTTGTGAATCCAAGTCCGTATATGGGTTATT	5000
Asp341Asn GAC-AAC	AATAACCCATATACGGACTTGGATTCACAACTCTTAGTGCTCTATA	5830
GAC-AAC	AACTTCAAAGGGGTTTGCAAATGTTCGCCGCTCAAAACGTTGACT CAGCACGATCTGAAATATGTCTCCTGCAAG	
	CATTTGCAAACCCCTTT	5831
	AAAGGGGTTTGCAAATG	5832
Trp Overproduction	GCTGCAGGAGACATATTTCAAATCGTTTTAAGTCAACGCTTTGAGA	5833
AS	GAAGAACATTTGCTAACCCATTTGAAGTGTACAGAGCATTAAGAAT	- J0000
Nicotiana tabacum	TGTGAATCCAAGCCCATATATGACTTACA	
Asp326Asn		

Asp326Asn GAC-AAC

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
	TGTAAGTCATATATGGGCTTGGATTCACAATTCTTAATGCTCTGTA CACTTCAAATGGGTTAGCAAATGTTCTTCTCCAAAGCGTTGACTT AAAACGATTTGAAATATGTCTCCTGCAGC	5834
	CATTTGCT <u>A</u> ACCCATTT	5835
	AAATGGGT <u>T</u> AGCAAATG	5836
Trp Overproduction AS Oryza sativa	CTAGCTGGTGACATTTTTCAAGTAGTCTTAAGCCAGCGTTTTGAGA GGCGTACATTTGCTAACCCCTTTGAGGTGTACCGTGCATTGCGTA TTGTCAATCCTAGTCCTTATATGGCCTATC	5837
Asp323Asn GAC-AAC	GATAGGCCATATAAGGACTAGGATTGACAATACGCAATGCACGGT ACACCTCAAAGGGGTTAGCAAATGTACGCCTCTCAAAACGCTGGC TTAAGACTACTTGAAAAATGTCACCAGCTAG	5838
	CATTTGCT <u>A</u> ACCCCTTT	5839
	AAAGGGGT <u>T</u> AGCAAATG	5840
Trp Overproduction AS Ruta graveolens	CTTGCTGGTGACATATTCCAGATCGTACTAAGTCAGCGTTTTGAAA GGCGAACGTTCGCAAACCCATTTGAAATCTATAGATCACTGAGGA TTGTTAATCCAAGCCCATATATGACTTATT	5841
Asp354Asn GAC-AAC	AATAAGTCATATATGGGCTTGGATTAACAATCCTCAGTGATCTATA GATTTCAAATGGGTTTGCGAACGTTCGCCTTTCAAAACGCTGACTT AGTACGATCTGGAATATGTCACCAGCAAG	5842
•	CGTTCGCAAACCCATTT	5843
	AAATGGGT <u>T</u> TGCGAACG	5844
Trp Overproduction AS Catharanthus roseus	CTGGCTGGGGACATATTCCAGCTTGTCCTAAGTCAGCGTTTTGAA CGGCGAACATTTGCAAATCCATTTGAAGTCTACCGAGCATTGAGA ATTGTCAACCCAAGTCCATATATGACTTATT	5845
Asp354Asn GAT-AAT	AATAAGTCATATATGGACTTGGGTTGACAATTCTCAATGCTCGGTA GACTTCAAATGGATTTGCAAATGTTCGCCGTTCAAAACGCTGACTT AGGACAAGCTGGAATATGTCCCCAGCCAG	5846
	CATITGCA <u>A</u> ATCCATTT	5847
	AAATGGAT <u>T</u> TGCAAATG	5848

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## Example 10

## Production of modified starch in plants

A principal aim of biotechnology is the improvement of crop plants for food value, agriculture, and to produce a range of plant-derived raw materials. Along with oils, fats and proteins, polysaccharides constitute the main raw materials derived from plants, and apart from cellulose, the storage polymer starch is the most important polysaccharide raw material. Starch is derived from a range of plants, but maize is the most important cultivated plant for the production of starch.

The polysaccharide starch is a polymer made up of glucose molecules. However, starch is not a homogeneous raw material and is, in fact, a highly complex mixture of various types of molecules which differ from each other, for example, in their degree of polymerization and in the degree of branching of the glucose chains. For example, amylose-starch is a basically non-branched polymer made up of  $\alpha$ -1,4-glycosidically branched glucose molecules, and amylopectin-starch is a complex mixture of variously branched glucose chains. The branching results from additional  $\alpha$ -1,6-glycosidic linkages. In plants from which starch is typically isolated, for example maize or potato, the starch is approximately 25% amylopectin-starch.

In maize, various mutants in starch metabolism are known, for example waxy, sugary, shrunken and opaque-2. In addition to producing a modified starch, these mutations greatly improve grain quality in maize, and thus expand the use of maize not only as the food but also for the important industrial materials in food chemistry. It would therefore be advantageous to be able readily to obtain mutants in these genes in particular maize genotypes as well as other plants. Such plants can be obtained, for example, using traditional breeding methods and through specific genetic modification by means of recombinant DNA techniques.

The attached tables disclose exemplary oligonucleotide base sequences which can be used to generate site-specific mutations in genes involved in starch metabolism.

Table 20
Genome-Altering Oligos Conferring Increased Starch

Phenotype, Gene, Plant & Targeted Atteration	Altering Oligos	SEQ III
Increased Starch ADPGPP Arabidopsis thaliana Ala99Lys GCA-AAA	GAACTTGAGACTGAGAAAAGGGATCCAAGGACAGTTGCTTCCATT ATTCTTGGAGGTGGAAAAGGAACTCGACTCTTTCCTCTCACAAAA CGCCGCGCCAAGCCTGCCGTTCCTATCGGGG	5849
	CCCCGATAGGAACGGCAGGCTTGGCGCGCGTTTTGTGAGAGGA AAGAGTCGAGTTCCTTTCCACCTCCAAGAATAATGGAAGCAACT GTCCTTGGATCCCTTTTCTCAGTCTCAAGTTC	5850
	GAGGTGGA <u>AA</u> AGGAACT	5851
	AGTTCCT <u>TT</u> TCCACCTC	5852
Increased Starch ADPGPP Arabidopsis thaliana Pro127Leu CCA-CTA	CAAAACGCCGCGCCAAGCCTGCCGTTCCTATCGGGGGAGCCTAT AGGTTGATAGATGTACTAATGAGCAATTGTATTAACAGCGGAATCA ACAAAGTCTACATACTCACACAATATAACTC	5853
	GAGTTATATTGTGTGAGTATGTAGACTTTGTTGATTCCGCTGTTAA TACAATTGCTCATTAGTACATCTATCAACCTATAGGCTCCCCCGAT AGGAACGCCAGGCTTGGCCGCGCGTTTTG	5854
	AGATGTAC <u>T</u> AATGAGCA	5855
	TGCTCATTAGTACATCT	5856
Increased Starch ADPGPP Arabidopsis thaliana Gly162Asn GGA-AAT	TCACACAATATAACTCAGCATCATTGAACAGGCATTTAGCCCGTGC TTACAACTCCAAT <u>AAT</u> CTTGGCTTTGGAGATGGCTATGTTGAGGTT CTTGCGGCCACTCAAACGCCAGGAGAATC	5857
	GATTCTCCTGGCGTTTGAGTGGCCGCAAGAACCTCAACATAGCCA TCTCCAAAGCCAAG <u>ATT</u> ATTGGAGTTGTAAGCACGGGCTAAATGC CTGTTCAATGATGCTGAGTTATATTGTGTGA	5858
	CTCCAAT <u>AAT</u> CTTGGCT	5859
	AGCCAAG <u>ATT</u> ATTGGAG	5860
Increased Starch ADPGPP Arabidopsis thaliana Gly162Asn GGA-AAC	TCACACATATAACTCAGCATCATTGAACAGGCATTTAGCCCGTGC TTACAACTCCAATAACCTTGGCTTTGGAGATGGCTATGTTGAGGTT CTTGCGGCCACTCAAACGCCAGGAGAATC	5861
	GATTCTCCTGGCGTTTGAGTGGCCGCAAGAACCTCAACATAGCCA TCTCCAAAGCCAAG <u>GTT</u> ATTGGAGTTGTAAGCACGGGCTAAATGC CTGTTCAATGATGCTGAGTTATATTGTGTGA	5862
	CTCCAAT <u>AAC</u> CTTGGCT	5863
	AGCCAAGGTTATTGGAG	5864

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID
Increased Starch ADPGPP Arabidopsis thaliana Asn100Lys AAT-AAA	GTTTGAGAGAAAGGTAGACCCGCAAAATGTGGCTGCAATCAT TCTAGGAGGAGGCAAAGGAGCTAAACTCTTCCCTCTTACAATGAG AGCCGCAACACCAGCTGTAAATATTCATCTT	5865
	AAGATGAATATTTACAGCTGGTGTTGCGGCTCTCATTGTAAGAGG GAAGAGTTTAGCTCCTTTGCCTCCTCCTAGAATGATTGCAGCCAC ATTTTGCGGGTCTACCTTTCTTCTCTCAAAC	5866
	GGAGGCAA <u>A</u> GGAGCTAA	5867
	TTAGCTCCTTTGCCTCC	5868
Increased Starch ADPGPP Arabidopsis thaliana	CTTGTGTCTTCAAATTATGTTAGGTTCCTGTTGGTGGATGCTACAG GCTGATCGATATCCTGATGAGTAACTGTATTAACAGCTGCATCAAC AAGATATTTGTGCTGACACAGTTCAACTC	5869
Pro128Leu CCG-CTG	GAGTTGAACTGTGTCAGCACAAATATCTTGTTGATGCAGCTGTTAA TACAGTTACTCATCAGGATATCGATCAGCCTGTAGCATCCACCAA CAGGAACCTAACATAATTTGAAGACACAAG	5870
	CGATATCCTGATGAGTA	5871
	TACTCATCAGGATATCG	5872
Increased Starch ADPGPP Arabidopsis thaliana	TGACACAGTTCAACTCAGCTTCCCTTAATCGACATTTAGCACGAAC TTATTTTGGGAAT <u>AAT</u> ATAAACTTTGGAGGTGGTTTCGTAGAGGTA CAAACACTATGACAATAATAACTCTCAGC	5873
GIy163Asn GGC-AAT	GCTGAGAGTTATTATTGTCATAGTGTTTGTACCTCTACGAAACCAC CTCCAAAGTTTAT <u>ATT</u> ATTCCCAAAATAAGTTCGTGCTAAATGTCG ATTAAGGGAAGCTGAGTTGAACTGTGTCA	5874
	TGGGAAT <u>AAT</u> ATAAACT	5875
	AGTITAT <u>ATT</u> ATTCCCA	5876
Increased Starch ADPGPP Arabidopsis thaliana	TGACACAGTTCAACTCAGCTTCCCTTAATCGACATTTAGCACGAAC TTATTTTGGGAAT <u>AAC</u> ATAAACTTTGGAGGTGGTTTCGTAGAGGTA CAAACACTATGACAATAATAACTCTCAGC	5877
Gly163Asn GGC-AAC	GCTGAGAGTTATTATTGTCATAGTGTTTGTACCTCTACGAAACCAC CTCCAAAGTTTATGTTATTCCCAAAATAAGTTCGTGCTAAATGTCG ATTAAGGGAAGCTGAGTTGAACTGTGTCA	5878
	TGGGAAT <b>AAC</b> ATAAACT	5879
	AGTITAT <u>GTT</u> ATTCCCA	5880
Increased Starch ADPGPP Lycopersicon	TTGAGGAACAACCAACGGCAGATCCAAAAGCTGTTGCCTCTGTCA TTCTAGGTGGTGGTAAAGGAACTCGTCTTTTTCCTCTTACAAGCA GAAGAGCTAAACCAGCTGTTCCTATTGGTGG	5881
esculentum Val94Lys GTT-AAA	CCACCAATAGGAACAGCTGGTTTAGCTCTTCTGCTTGTAAGAGGA AAAAGACGAGTTCC <u>TTT</u> ACCACCACCTAGAATGACAGAGGCAACA GCTTTTGGATCTGCCGTTGGTTGTTCCTCAA	5882
	TGGTGGT <u>AAA</u> GGAACTC	5883

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
	GAGTTCC <u>TTT</u> ACCACCA	5884
Increased Starch ADPGPP Lycopersicon	CAAGCAGAAGAGCTAAACCAGCTGTTCCTATTGGTGGTTGTTACC GGCTAATTGATGTACAAATGAGTAACTGCATTAACAGTGGCATAC GGAAAATTTTCATCTTAACACAGTTCAATTC	5885
esculentum Pro122Leu CCA-CAA	GAATTGAACTGTGTTAAGATGAAAATTTTCCGTATGCCACTGTTAA TGCAGTTACTCATTTGTACATCAATTAGCCGGTAACAACCACCAAT AGGAACAGCTGGTTTAGCTCTTCTGCTTG	5886
	TGATGTAC <u>A</u> AATGAGTA	5887
	TACTCATT <u>T</u> GTACATCA	5888
Increased Starch ADPGPP Lycopersicon	CACAGTTCAATTCCTTTTCCCTCAATCGTCACCTTGCCCGCACGTA TAATTTTGGAAATAATGTGGGGTTTTGGAGATGGATTTGTGGAGGTT TTAGCTGCAACCCAGACTCCAGGGGATGC	5889
esculentum Gly158Asn GGA-AAT	GCATCCCTGGAGTCTGGGTTGCAGCTAAAACCTCCACAAATCCA TCTCCAAAACCCAC <u>ATT</u> ATTTCCAAAATTATACGTGCGGGCAAGGT GACGATTGAGGGAAAAGGAATTGAACTGTG	5890
	TGGAAAT <u>AAT</u> GTGGGTT	5891
	AACCCAC <u>ATT</u> ATTTCCA	5892
Increased Starch ADPGPP Lycopersicon	CACAGTTCAATTCCTTTTCCCTCAATCGTCACCTTGCCCGCACGTA TAATTTTGGAAATAACGTGGGTTTTTGGAGATGGATTTGTGGAGGTT TTAGCTGCAACCCAGACTCCAGGGGATGC	5893
esculentum Gly158Asn GGA-AAC	GCATCCCTGGAGTCTGGGTTGCAGCTAAAACCTCCACAAATCCA TCTCCAAAACCCACGTTATTTCCAAAATTATACGTGCGGGCAAGGT GACGATTGAGGGAAAAGGAATTGAACTGTG	5894
	TGGAAAT <u>AAC</u> GTGGGTT	5895
·	AACCCAC <u>GTT</u> ATTTCCA	5896
Increased Starch ADPGPP Cicer arietinum	ACGTAGATTTGGAAAAAAGAGACCCAAGTACAGTTGTAGCAATTAT ACTAGGTGGAGGT <u>AAA</u> GGAACTCGTCTCTTCCCTCTCACCAAGC GACGAGCCAAGCCTGCTGTTCCAATTGGAGG	5897
Ala101Lys GCT-AAA	CCTCCAATTGGAACAGCAGGCTTGGCTCGCTTGGTGAGAGG GAAGAGACGAGTTCC <u>TTT</u> ACCTCCACCTAGTATAATTGCTACAACT GTACTTGGGTCTCTTTTTTCCAAATCTACGT	5898
	TGGAGGT <u>AAA</u> GGAACTC	5899
	GAGTTCCTTTACCTCCA	5900

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Increased Starch ADPGPP Cicer arietinum Pro129Leu CCA-CTA	CCAAGCGACGAGCCAAGCCTGCTGTTCCAATTGGAGGTGCTTATA GGCTGATAGATGTACTAATGAGTAACTGCATCAATAGTGGGATCAA CAAAGTATACATTCTCACTCAATTTAATTC	5901
	GAATTAAATTGAGTGAGAATGTATACTTTGTTGATCCCACTATTGAT GCAGTTACTCATTAGTACATCTATCAGCCTATAAGCACCTCCAATT GGAACAGCAGGCTTGGCTCGCTTGG	5902
	AGATGTAC <u>T</u> AATGAGTA	5903
	TACTCATTAGTACATCT	5904
Increased Starch ADPGPP Cicer arietinum	CTCAATTTAATTCAGCCTCACTCAACAGGCATATTGCACGTGCTTA TAACTCTGGTACT <u>AAT</u> GTCACTTTTGGAGATGGCTATGTTGAGGTT CTTGCAGCAACTCAAACTCCAGGGGAGCA	5905
Gly165Asn GGA-AAT	TGCTCCCTGGAGTTTGAGTTGCTGCAAGAACCTCAACATAGCCA TCTCCAAAAGTGACATTAGTACCAGAGTTATAAGCACGTGCAATAT GCCTGTTGAGTGAGGCTGAATTAAATTGAG	5906
	TGGTACTAATGTCACTT	5907
	AAGTGAC <u>ATT</u> AGTACCA	5908
Increased Starch ADPGPP Cicer arietinum	CTCAATTTAATTCAGCCTCACTCAACAGGCATATTGCACGTGCTTA TAACTCTGGTACTAACGTCACTTTTTGGAGATGGCTATGTTGAGGTT CTTGCAGCAACTCAAACTCCAGGGGAGCA	5909
Gly165Asn GGA-AAC	TGCTCCCTGGAGTTTGAGTTGCTGCAAGAACCTCAACATAGCCA TCTCCAAAAGTGACGTTAGTACCAGAGTTATAAGCACGTGCAATAT GCCTGTTGAGTGAGGCTGAATTAAATTGAG	5910
	TGGTACT <u>AAC</u> GTCACTT	5911
	AAGTGAC <u>GTT</u> AGTACCA	5912
Increased Starch ADPGPP Ipomoea batatas	ATATTGGAGAGGCGTCGGGCAAACCCTAAGAATGTGGCTGCAATC ATACTGCCAGGCGGTAAAGGGACACCCTATTCCCTCTCACCAAT CGAGCTGCAACCCCTGCTGTTCCACTTGGAG	5913
Ala94Lys GCA-AAA	CTCCAAGTGGAACAGCAGGGGTTGCAGCTCGATTGGTGAGAGGG AATAGGTGTGCCCT <u>TT</u> ACCGCCTGGCAGTATGATTGCAGCCACA TTCTTAGGGTTTGCCCGACGCCTCTCCAATAT	5914
	CAGGCGGT <u>AA</u> AGGGACA	5915
	TGTCCCTTTACCGCCTG	5916
Increased Starch ADPGPP Ipomoea batatas	CCAATCGAGCTGCAACCCCTGCTGTTCCACTTGGAGGATGCTATA GGTTGATCGACATTCTAATGAGCAACTGCATCAACAGCGGGGTTA ACAAGATCTTTGTGCTGACCCAGTTCAATTC	5917
Pro122Leu CCA-CTA	GAATTGAACTGGGTCAGCACAAAGATCTTGTTAACCCCGCTGTTG ATGCAGTTGCTCATTAGAATGTCGATCAACCTATAGCATCCTCCAA GTGGAACAGCAGGGGTTGCAGCTCGATTGG	5918
	CGACATTC <u>T</u> AATGAGCA	5919

Phenotype, Gene, Plant & Targeted Afteration	Aftering Oligos	SEQID No:
	TGCTCATTAGAATGTCG	5920
Increased Starch ADPGPP Ipomoea batatas Gly157Asn GGT-AAT	TGACCCAGTTCAATTCAGCTTCTCTTAACCGTCACATTTCCCGTAC CGTCTTTGGCAAT <u>AAT</u> GTGAGCTTCGGAGATGGATTTGTTGAGGT GCTGGCTGCAACCCAAACACAAGGGGAAAC	5921
	GTTTCCCCTTGTGTTTGGGTTGCAGCCAGCACCTCAACAAATCCA TCTCCGAAGCTCACATTATTGCCAAAGACGGTACGGGAAATGTGA CGGTTAAGAGAAGCTGAATTGAACTGGGTCA	5922
,	TGGCAAT <u>AAT</u> GTGAGCT	5923
	AGCTCAC <u>ATT</u> ATTGCCA	5924
Increased Starch ADPGPP Ipomoea batatas	TGACCCAGTTCAATTCAGCTTCTCTTAACCGTCACATTTCCCGTAC CGTCTTTGGCAAT <u>AAC</u> GTGAGCTTCGGAGATGGATTTGTTGAGGT GCTGGCTGCAACCCAAACACAAGGGGAAAC	5925
Gly157Asn GGT-AAC	GTTTCCCCTTGTGTTTGGGTTGCAGCCAGCACCTCAACAAATCCA TCTCCGAAGCTCACGTTATTGCCAAAGACGGTACGGGAAATGTGA CGGTTAAGAGAAGCTGAATTGAACTGGGTCA	5926
	TGGCAAT <u>AAC</u> GTGAGCT	5927
	AGCTCAC <u>GTT</u> ATTGCCA	5928
Increased Starch ADPGPP Oryza sativa	CATTCCGGAGGAACTTTGCGGATCCAAATGAGGTTGCTGCTGTTA TATTGGGTGGTGGCAAAGGGACTCAACTTTTTCCTCTCACAAGCA CAAGGGCCACGCCTGCTGTTCCTATTGGAGG	5929
Thr96Lys ACC-AAA	CCTCCAATAGGAACAGCAGGCGTGGCCCTTGTGCTTGTGAGAGG AAAAAGTTGAGTCCCTTTGCCACCACCCAATATAACAGCAGCAAC CTCATTTGGATCCGCAAAGTTCCTCCGGAATG	5930
	TGGTGGCA <u>AA</u> GGGACTC	5931
	GAGTCCC <u>TT</u> TGCCACCA	5932
Increased Starch ADPGPP Oryza sativa	CAAGCACAAGGGCCACGCCTGCTGTTCCTATTGGAGGATGCTATA GGCTTATCGATATCCTCATGAGCAACTGTTTCAACAGTGGCATAAA CAAGATATTCATAATGACTCAATTCAACTC	5933
Pro124Leu CCC-CTC	GAGTTGAATTGAGTCATTATGAATATCTTGTTTATGCCACTGTTGAA ACAGTTGCTCATGAGGATATCGATAAGCCTATAGCATCCTCCAATA GGAACAGCAGGCGTGGCCCTTGTGCTTG	5934
	CGATATCCTCATGAGCA	5935
	TGCTCATGAGGATATCG	5936
Increased Starch ADPGPP Oryza sativa	TGACTCAATTCAACTCAGCATCTCTTAATCGTCACATTCATCGTAC GTACCTTGGTGGTAATATCAACTTTACTGATGGTTCTGTTGAGGTA TTAGCCGCTACACAAATGCCTGGGGAGGC	5937
Gly159Asn GGA-AAT	GCCTCCCAGGCATTTGTGTAGCGGCTAATACCTCAACAGAACCA TCAGTAAAGTTGAT <u>ATT</u> ACCACCAAGGTACGTACGATGAATGTGAC GATTAAGAGATGCTGAGTTGAATTGAGTCA	5938

Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID NO:
	TGGTGGT <u>AAT</u> ATCAACT	5939
	AGTTGAT <u>ATT</u> ACCACCA	5940
Increased Starch ADPGPP Onyza sativa	TGACTCAATTCAACTCAGCATCTCTTAATCGTCACATTCATCGTAC GTACCTTGGTGGTAACACTCTACTGATGGTTCTGTTGAGGTA TTAGCCGCTACACAAATGCCTGGGGAGGC	5941
Gly159Asn GGA-AAC	GCCTCCCAGGCATTTGTGTAGCGGCTAATACCTCAACAGAACCA TCAGTAAAGTTGAT <u>GTT</u> ACCACCAAGGTACGTACGATGAATGTGA CGATTAAGAGATGCTGAGTTGAATTGAGTCA	5942
	TGGTGGT <u>AAC</u> ATCAACT	5943
	AGTTGATGTTACCACCA	5944
Increased Starch ADPGPP Triticum aestivum	GTCCTTCAGGAGGATTAAGCGATCCGAACGAGGTTGCGGCCGTC ATACTCGGCGGCGGCA <u>AA</u> GGGACTCAGCTCTTCCCACTCACGAG CACAAGGGCCACACCTGCTGTTCCTATTGGAGG	5945
Thr80Lys ACC-AAA	CCTCCAATAGGAACAGCAGGTGTGGCCCTTGTGCTCGTGAGTGG GAAGAGCTGAGTCCCTTTGCCGCCGCCGAGTATGACGGCCGCAA CCTCGTTCGGATCGCTTAATCCTCCTGAAGGAC	5946
	CGGCGGCA <u>AA</u> GGGACTC	5947
	GAGTCCC <u>TT</u> TGCCGCCG	5948
Increased Starch ADPGPP Triticum aestivum	CGAGCACAAGGGCCACACCTGCTGTTCCTATTGGAGGATGTTACA GGCTCATCGACATTCTCATGAGCAACTGCTTCAACAGTGGCATCA ACAAGATATTCGTCATGACCCAGTTCAACTC	5949
Pro108Leu CCC-CTC	GAGTTGAACTGGGTCATGACGAATATCTTGTTGATGCCACTGTTG AAGCAGTTGCTCATGAGAATGTCGATGAGCCTGTAACATCCTCCA ATAGGAACAGCAGGTGTGGCCCTTGTGCTCG	5950
	CGACATTC <u>T</u> CATGAGCA	5951
	TGCTCATGAGAATGTCG	5952
Increased Starch ADPGPP Triticum aestivum Gly143Asn GGA-AAT	TGACCCAGTTCAACTCGGCCTCCCTTAATCGTCACATTCACCGCA CCTACCTCGGCGGGAATATCAATTTCACTGATGGATCCGTTGAGG TATTGGCCGCGACGCAAATGCCCGGGGAGGC	5953
	GCCTCCCGGGCATTTGCGTCGCGGCCAATACCTCAACGGATCC ATCAGTGAAATTGAT <u>ATT</u> CCCGCCGAGGTAGGTGCGGTGAATGTG ACGATTAAGGGAGGCCGAGTTGAACTGGGTCA	5954
	CGGCGGG <u>AAT</u> ATCAATT	5955
	AATTGAT <u>ATT</u> CCCGCCG	5956
Increased Starch ADPGPP Triticum aestivum Gly143Asn	TGACCCAGTTCAACTCGGCCTCCCTTAATCGTCACATTCACCGCA CCTACCTCGGCGGGAACATCAATTTCACTGATGGATCCGTTGAGG TATTGGCCGCGACGCAAATGCCCGGGGAGGC	5957

Gly143Asn GGA-AAC

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	GCCTCCCGGGCATTTGCGTCGCGGCCAATACCTCAACGGATCC ATCAGTGAAATTGATGTTCCCGCCGAGGTAGGTGCGGTGAATGTG ACGATTAAGGGAGGCCGAGTTGAACTGGGTCA	5958
	CGGCGGGAACATCAATT	5959
	AATTGAT <u>GTT</u> CCCGCCG	5960
Increased Starch ADPGPP Oryza sativa	CCTCCCGAAAGAATTATGCTGATGCAAGCCACGTTTCTGCTGTCA TTTTGGGTGGAGGCAAAGGAGTTCAACTCTTTCCTCTGACAAGCA CAAGGGCTACCCCGCTGTTCCTGTTGGAGG	5961
Thr95Lys ACT-AAA	CCTCCAACAGGAACAGCGGGGGTAGCCCTTGTGCTTGTCAGAGG AAAGAGTTGAACTCCTTTGCCTCCACCCAAAATGACAGCAGAAAC GTGGCTTGCATCAGCATAATTCTTTCGGGAGG	5962
	TGGAGGCA <u>AA</u> GGAGTTC	5963
	GAACTCC <u>TT</u> TGCCTCCA	5964
Increased Starch ADPGPP Oryza sativa	CAAGCACAAGGGCTACCCCCGCTGTTCCTGTTGGAGGATGTTACA GGCTTATTGACATCCTTATGAGCAATTGCTTCAATAGCGGAATAAA TAAAATATTTGTGATGACTCAGTTCAATTC	5965
Pro123Leu CCT-CTT	GAATTGAACTGAGTCATCACAAATATTTTATTTATTCCGCTATTGAA GCAATTGCTCATAAGGATGTCAATAAGCCTGTAACATCCTCCAACA GGAACAGCGGGGGTAGCCCTTGTGCTTG	5966
	TGACATCC <u>T</u> TATGAGCA	5967
	TGCTCATA <u>A</u> GGATGTCA	5968
Increased Starch ADPGPP Oryza sativa	TGACTCAGTTCAATTCTGCTTCTCTTAATCGCCATATCCATCATAC ATACCTTGGTGGGAATATCAACTTTACTGATGGGTCTGTGCAGGT ATTGGCTGCTACACAAATGCCTGACGAACC	5969
Gly158Asn GGG-AAT	GGTTCGTCAGGCATTTGTGTAGCAGCCAATACCTGCACAGACCCA TCAGTAAAGTTGAT <u>ATT</u> CCCACCAAGGTATGTATGATGGATATGGC GATTAAGAGAAGCAGAATTGAACTGAGTCA	5970
	TGGTGGG <u>AAT</u> ATCAACT	597.1
	AGTTGAT <u>ATT</u> CCCACCA	5972
Increased Starch ADPGPP Onyza sativa	TGACTCAGTTCAATTCTGCTTCTCTTAATCGCCATATCCATCATAC ATACCTTGGTGGGAACATCAACTTTACTGATGGGTCTGTGCAGGT ATTGGCTGCTACACAAATGCCTGACGAACC	5973
Gly158Asn GGG-AAC	GGTTCGTCAGGCATTTGTGTAGCAGCCCAATACCTGCACAGACCCA TCAGTAAAGTTGATGTTCCCACCAAGGTATGTATGATGGATATGGC GATTAAGAGAAGCAGAATTGAACTGAGTCA	5974
	TGGTGGG <u>AAC</u> ATCAACT	5975
	AGTTGATGTTCCCACCA	5976

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Increased Starch ADPGPP Triticum aestivum	CCTTCCGCAGGAATTACGCCGATCCGAACGAGGTCGCGGCCGTC ATACTCGGCGGTGGCAAAGGGACTCAGCTCTTCCCTCTCACAAG CACAAGGGCCACACCTGCTGTTCCTATTGGAGG	5977
Thr99Lys ACC-AAA	CCTCCAATAGGAACAGCAGGTGTGGCCCTTGTGCTTGTGAGAGG GAAGAGCTGAGTCCCTTTGCCACCGCCGAGTATGACGGCCGCGA CCTCGTTCGGATCGGCGTAATTCCTGCGGAAGG	5978
1	CGGTGGCA <u>AA</u> GGGACTC	5979
	GAGTCCCTTTGCCACCG	5980
Increased Starch ADPGPP Triticum aestivum	CAAGCACAAGGGCCACACCTGCTGTTCCTATTGGAGGATGTTACA GGCTCATCGATATTCTCATGAGCAACTGCTTCAATAGTGGCATCAA CAAGATATTCGTCATGACGCAGTTCAACTC	5981
Pro127Leu CCC-CTC	GAGTTGAACTGCGTCATGACGAATATCTTGTTGATGCCACTATTGA AGCAGTTGCTCATGAGAATATCGATGAGCCTGTAACATCCTCCAA TAGGAACAGCAGGTGTGGCCCTTGTGCTTG	5982
	CGATATTC <u>T</u> CATGAGCA	5983
	TGCTCATG <u>A</u> GAATATCG	5984
Increased Starch ADPGPP Triticum aestivum	TGACGCAGTTCAACTCGGCCTCTCTTAATCGTCACATTCACCGCA CCTACCTCGGCGGGAATATCAATTTCACTGATGGATCTGTTGAGG TATTGGCCGCGACGCAAATGCCCGGGGAGGC	5985
Gly162Asn GGA-AAT	GCCTCCCGGGCATTTGCGTCGCGGCCAATACCTCAACAGATCC ATCAGTGAAATTGAT <u>ATT</u> CCCGCCGAGGTAGGTGCGGTGAATGTG ACGATTAAGAGAGGCCGAGTTGAACTGCGTCA	5986
	CGGCGGG <u>AAT</u> ATCAATT	5987
	AATTGAT <u>ATT</u> CCCGCCG	5988
Increased Starch ADPGPP Triticum aestivum	TGACGCAGTTCAACTCGGCCTCTCTTAATCGTCACATTCACCGCA CCTACCTCGGCGGGAACATCAATTTCACTGATGGATCTGTTGAGG TATTGGCCGCGACGCAAATGCCCGGGGAGGC	5989
Gly162Asn GGA-AAC	GCCTCCCGGGCATTTGCGTCGCGGCCAATACCTCAACAGATCC ATCAGTGAAATTGATGTTCCCGCCGAGGTAGGTGCGGTGAATGTG ACGATTAAGAGAGGCCGAGTTGAACTGCGTCA	5990
	CGGCGGGAACATCAATT	5991
	AATTGAT <u>GTT</u> CCCGCCG	5992
Increased Starch ADPGPP Zea mays	CTTTTCGGAGGAATTATGCTGATCCTAATGAAGTCGCTGCCGTCA TTTTGGGTGGTGGTAAAGGGGACTCAGCTTTTCCCTCTCACAAGCA CAAGGGCCACCCCTGCTGTTCCTATTGGAGG	5993
Thr96Lys ACC-AAA	CCTCCAATAGGAACAGCAGGGGTGGCCCTTGTGCTTGTGAGAGG GAAAAGCTGAGTCCCTTACCACCACCAAAATGACGGCAGCGAC TTCATTAGGATCAGCATAATTCCTCCGAAAAG	5994
	TGGTGGTA <u>AA</u> GGGACTC	5995

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	GAGTCCCTTTACCACCA	5996
Increased Starch ADPGPP Zea mays	CAAGCACAAGGGCCACCCCTGCTGTTCCTATTGGAGGATGTTACA GGCTTATTGATATCCTCATGAGCAACTGTTTCAACAGTGGCATAAA CAAGATATTTGTTATGACTCAGTTCAACTC	5997
Pro124Leu CCC-CTC	GAGTTGAACTGAGTCATAACAAATATCTTGTTTATGCCACTGTTGA AACAGTTGCTCATGAGGATATCAATAAGCCTGTAACATCCTCCAAT AGGAACAGCAGGGGTGGCCCTTGTGCTTG	5998
	TGATATCCTCATGAGCA	5999
	TGCTCATGAGGATATCA	6000
Increased Starch ADPGPP Zea mays	TGACTCAGTTCAACTCAGCTTCTCTTAACCGTCACATTCATCGTAC CTATCTTGGTGGGAATATCAACTTCACTGATGGATCTGTTGAGGT GCTGGCTGCAACACAAATGCCTGGGGAGGC	6001
Gly159Asn GGG-AAT	GCCTCCCAGGCATTTGTGTTGCAGCCAGCACCTCAACAGATCCA TCAGTGAAGTTGAT <u>ATT</u> CCCACCAAGATAGGTACGATGAATGTGA CGGTTAAGAGAAGCTGAGTTGAACTGAGTCA	6002
	TGGTGGG <u>AAT</u> ATCAACT	6003
	AGTTGAT <u>ATT</u> CCCACCA	6004
Increased Starch ADPGPP Zea mays	TGACTCAGTTCAACTCAGCTTCTCTTAACCGTCACATTCATCGTAC CTATCTTGGTGGGAACATCAACTTCACTGATGGATCTGTTGAGGT GCTGGCTGCAACACAAATGCCTGGGGAGGC	6005
Gly159Asn GGG-AAC	GCCTCCCAGGCATTTGTGTTGCAGCCAGCACCTCAACAGATCCA TCAGTGAAGTTGAT <u>GTT</u> CCCACCAAGATAGGTACGATGAATGTGA CGGTTAAGAGAAGCTGAGTTGAACTGAGTCA	6006
	TGGTGGG <u>AAC</u> ATCAACT	6007
	AGTTGAT <u>GTT</u> CCCACCA	6008
Increased Starch ADPGPP Solanum tuberosum	CTTGAGAGGCAAAAGAAGGGCGATGCAAGGACAGTAGTAGCAAT CATTCTAGGAGGGGGAAAGGGAACTCGTCTTTTCCCCCTCACCAA ACGTCGTGCTAAGCCTGCCGTTCCAATGGGAG	6009
Ala58Lys GCG-AAG	CTCCCATTGGAACGCCAGGCTTAGCACGACGTTTGGTGAGGGGG AAAAGACGAGTTCCC <u>TT</u> TCCCCCTCCTAGAATGATTGCTACTACTG TCCTTGCATCGCCCTTCTTTTGCCTCTCAAG	6010
	GAGGGGAACT	6011
	AGTTCCC <u>TT</u> TCCCCCTC	6012
Increased Starch ADPGPP Solanum tuberosum	CCAAACGTCGTGCTAAGCCTGCCGTTCCAATGGGAGGAGCATATA GGCTAATTGATGTACTAATGAGCAACTGTATTAACAGTGGCATCAA CAAAGTATACATTCTCACTCAATTCAACTC	6013
Pro86Leu CCA-CTA	GAGTTGAATTGAGTGAGAATGTATACTTTGTTGATGCCACTGTTAA TACAGTTGCTCATTAGTACATCAATTAGCCTATATGCTCCCAT TGGAACGGCAGGCTTAGCACGACGTTTGG	6014

Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQID No:
	TGATGTAC <u>T</u> AATGAGCA	6015
	TGCTCATT <u>A</u> GTACATCA	6016
Increased Starch ADPGPP Solanum tuberosum	CTCAATTCAACTCAGCCTCACTTAACAGGCATATAGCTCGTGCTTA CAACTTTGGCAAT <u>AAT</u> GTCACATTCGAGAGTGGCTATGTCGAGGT CTTAGCAGCAACTCAAACACCAGGTGAATT	6017
GIy122Asn GGG-AAT	AATTCACCTGGTGTTTGAGTTGCTGACAAGACCTCGACATAGCCA CTCTCGAATGTGACATTATTGCCAAAGTTGTAAGCACGAGCTATAT GCCTGTTAAGTGAGGCTGAGTTGAATTGAG	6018
	TGGCAAT <u>AAT</u> GTCACAT	6019
	ATGTGAC <u>ATT</u> ATTGCCA	6020
Increased Starch ADPGPP Solanum tuberosum	CTCAATTCAACTCAGCCTCACTTAACAGGCATATAGCTCGTGCTTA CAACTTTGGCAAT <u>AAC</u> GTCACATTCGAGAGTGGCTATGTCGAGGT CTTAGCAGCAACTCAAACACCAGGTGAATT	6021
Gly122Asn GGG-AAC	AATTCACCTGGTGTTTGAGTTGCTGACAAGACCTCGACATAGCCA CTCTCGAATGTGAC <u>GTT</u> ATTGCCAAAGTTGTAAGCACGAGCTATAT GCCTGTTAAGTGAGGCTGAGTTGAATTGAG	6022
	TGGCAAT <u>AAC</u> GTCACAT	6023
	ATGTGAC <u>GTT</u> ATTGCCA	6024
Increased Starch ADPGPP Beta vulgaris	TATTTGAATCTCCAAAAGCTGACCCAAAAAATGTGGCTGCAATTGT GCTGGGTGGTGAAAGGGACTCGCCTCTTTCCTCTTACTAGCA GGAGAGCTAAGCCAGCAGTGCCAATTGGAGG	6025
Ala98Lys GCT-AAA	CCTCCAATTGGCACTGCTGGCTTAGCTCCTGCTAGTAAGAGGA AAGAGGCGAGTCCC <u>TTT</u> ACCACCACCAGCACAATTGCAGCCACA TTTTTTGGGTCAGCTTTTGGAGATTCAAATA	6026
	TGGTGGT <u>AAA</u> GGGACTC	6027
	GAGTCCC <u>TTT</u> ACCACCA	6028
Increased Starch ADPGPP Beta vulgaris	TATTTGAATCTCCAAAAGCTGACCCAAAAAATGTGGCTGCAATTGT GCTGGGTGGTGACGGGGACTCGCCTCTTTCCTCTTACTAGCA GGAGAGCTAAGCCAGCAGTGCCAATTGGAGG	6029
Ala98Lys GCT-AAC	CCTCCAATTGGCACTGCTGGCTTAGCTCTCCTGCTAGTAAGAGGA AAGAGGCGAGTCCCGTTACCACCACCCAGCACAATTGCAGCCAC ATTTTTTGGGTCAGCTTTTGGAGATTCAAATA	6030
	TGGTGGT <u>AAC</u> GGGACTC	6031
	GAGTCCC <u>GTT</u> ACCACCA	6032
Increased Starch ADPGPP Beta vulgaris Pro126Leu	CTAGCAGGAGAGCTAAGCCAGCAGTGCCAATTGGAGGGTGTTAC AGGCTGATTGATGTGCTTATGAGCAACTGCATCAACAGTGGCATT AGAAAGATTTTCATTCTTACCCAGTTCAATTC	6033

Pro126Leu CCT-CTT

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	GAATTGAACTGGGTAAGAATGAAAATCTTTCTAATGCCACTGTTGA TGCAGTTGCTCATAAGCACATCAATCAGCCTGTAACACCCTCCAA TTGGCACTGCTGGCTTAGCTCTCCTGCTAG	6034
	TGATGTGC <u>T</u> TATGAGCA TGCTCATA <u>A</u> GCACATCA	6035 6036
Increased Starch ADPGPP Beta vulgaris	CCCAGTTCAATTCGTTTTCGCTTAATCGTCATCTTGCTCGAACCTA TAATTTTGGAGATATGTGAATTTTGGGGATGGCTTTGTGGAGGTT TTTGCTGCTACACAAACACCTGGAGAATC	6037
Gly162Asn GGT-AAT	GATTCTCCAGGTGTTTGTGTAGCAGCAAAAACCTCCACAAAGCCA TCCCCAAAATTCACATTATCTCCAAAATTATAGGTTCGAGCAAGAT GACGATTAAGCGAAAACGAATTGAACTGGG	6038
	TGGAGAT <u>AAT</u> GTGAATT	6039
	AATTCAC <u>ATT</u> ATCTCCA	6040
Increased Starch ADPGPP Beta vulgaris	CCCAGTTCAATTCGTTTTCGCTTAATCGTCATCTTGCTCGAACCTA TAATTTTGGAGATAACGTGAATTTTGGGGGATGGCTTTGTGGAGGT TTTTGCTGCTACACAAACACCCTGGAGAATC	6041
Gly162Asn GGT-AAC	GATTCTCCAGGTGTTTGTGTAGCAGCAAAACCTCCACAAAGCCA TCCCCAAAATTCACGTTATCTCCAAAATTATAGGTTCGAGCAAGAT GACGATTAAGCGAAAACGAATTGAACTGGG	6042
	TGGAGAT <u>AAC</u> GTGAATT	6043
	AATTCAC <u>GTT</u> ATCTCCA	6044

Table 21
Oligonucleotides to produce plants with waxy starch

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID No:
Waxy starch GBSS Arabidopsis thaliana	GAATCCAGGTAAACGGGTAGTTCATAATGGCAACTGTGACTGCTTC TTCTAACTTTGTGTGAAGAACTTCACTTTTCAACAATCATGGTGCTT CTTCATGCTCTGATGTCGCTCAGATTAC	6045
Ser12Term TCA-TGA	GTAATCTGAGCGACATCAGAGCATGAAGAAGCACCATGATTGTTGA AAAGTGAAGTTCTT <u>C</u> ACACAAAGTTAGAAGAAGCAGTCACAGTTGC CATTATGAACTACCCGTTTACCTGGATTC	6046
	CTTTGTGT <u>G</u> AAGAACTT	6047
	AAGTTCTT <u>C</u> ACACAAAG	6048
Waxy starch GBSS Arabidopsis thaliana	ATCCAGGTAAACGGGTAGTTCATAATGGCAACTGTGACTGCTTCTT CTAACTTTGTGTCATGAACTTCACTTTTCAACAATCATGGTGCTTCT TCATGCTCTGATGTCGCTCAGATTACCT	6049
Arg13Term AGA-TGA	AGGTAATCTGAGCGACATCAGAGCATGAAGAAGCACCATGATTGTT GAAAAGTGAAGTTCAATGACACAAAGTTAGAAGAAGCAGTCACAGTT GCCATTATGAACTACCCGTTTACCTGGAT	6050
	TTGTGTCATGAACTTCA	6051
	TGAAGTTC <u>A</u> TGACACAA	6052
Waxy starch GBSS Arabidopsis thaliana	TAAACGGGTAGTTCATAATGGCAACTGTGACTGCTTCTTCTAACTT TGTGTCAAGAACTTGACTTTTCAACAATCATGGTGCTTCTTCATGCT CTGATGTCGCTCAGATTACCTTAAAAGG	6053
Ser15Term TCA-TGA	CCTTTTAAGGTAATCTGAGCGACATCAGAGCATGAAGAAGCACCAT GATTGTTGAAAAGTCAAGTTCTTGACACAAAGTTAGAAGAAGCAGT CACAGTTGCCATTATGAACTACCCGTTTA	6054
	AAGAACTT <u>G</u> ACTTTTCA	6055
	TGAAAAGT <u>C</u> AAGTTCTT	6056
Waxy starch GBSS Arabidopsis thaliana	TGACTGCTTCTTCTAACTTTGTGTCAAGAACTTCACTTTTCAACAAT CATGGTGCTTCTTGATGCTCTGATGTCGCTCAGATTACCTTAAAAG GCCAATCCTTGACTCATTGTGGGTTAAG	6057
Ser24Term TCA-TGA	CTTAACCCACAATGAGTCAAGGATTGGCCTTTTAAGGTAATCTGAG CGACATCAGAGCATCAAGAAGCACCATGATTGTTGAAAAGTGAAGT TCTTGACACAAAGTTAGAAGAAGCAGTCA	6058
	TGCTTCTT <u>G</u> ATGCTCTG	6059
_	CAGAGCAT <u>C</u> AAGAAGCA	6060

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID No:
Waxy starch GBSS Arabidopsis thaliana	TGCTTCTTCTAACTTTGTGTCAAGAACTTCACTTTTCAACAATCATG GTGCTTCTTCATGATCTGATGTCGCTCAGATTACCTTAAAAGGCCA ATCCTTGACTCATTGTGGGTTAAGGTCA	6061
Cys25Term TGC-TGA	TGACCTTAACCCACAATGAGTCAAGGATTGGCCTTTTAAGGTAATC TGAGCGACATCAGATCATGAAGAAGCACCATGATTGTTGAAAAAGTG AAGTTCTTGACACAAAGTTAGAAGAAGCA	6062
	TCTTCATGATCTGATGT	6063
	ACATCAGA <u>T</u> CATGAAGA	6064
Waxy starch GBSS Antirrhinum majus	GTAACAGCTTCACAGTTGGTGTCACATGTCCATGGTGGAGCAACG TCTTCACCGGATACTTAAACAAACTTGGCCCAGGTTGGCCTCAGG AACCAGCAATTCACTCACAATGGGTTGAGAT	6065
Lys24Term AAA-TAA	ATCTCAACCCATTGTGAGTGAATTGCTGGTTCCTGAGGCCAACCT GGGCCAAGTTTGTTTAAGTATCCGGTGAAGACGTTGCTCCACCAT GGACATGTGACACCAACTGTGAAGCTGTTAC	6066
	CGGATACT <u>T</u> AAACAAAC	6067
<u> </u>	GTTTGTTT <u>A</u> AGTATCCG	6068
Waxy starch GBSS Antirrhinum majus	CACAGTTGGTGTCACATGTCCATGGTGGAGCAACGTCTTCACCGG ATACTAAAACAAACTAGGCCCAGGTTGGCCTCAGGAACCAGCAAT TCACTCACAATGGGTTGAGATCAATAAACAT	6069
Leu27Term TTG-TAG	ATGITTATTGATCTCAACCCATTGTGAGTGAATTGCTGGTTCCTGA GGCCAACCTGGGCCTAGTTTGTTTTAGTATCCGGTGAAGACGTTG CTCCACCATGGACATGTGACACCAACTGTG	6070
	AACAAACT <u>A</u> GGCCCAGG	6071
	CCTGGGCC <u>T</u> AGTTTGTT	6072
Waxy starch GBSS Antirrhinum majus	TTGGTGTCACATGTCCATGGTGGAGCAACGTCTTCACCGGATACT AAAACAAACTTGGCCTAGGTTGGCCTCAGGAACCAGCAATTCACT CACAATGGGTTGAGATCAATAAACATGGTTG	6073
Gin29Term CAG-TAG	CAACCATGTTTATTGATCTCAACCCATTGTGAGTGAATTGCTGGTT CCTGAGGCCAACCTAGGCCAAGTTTGTTTTAGTATCCGGTGAAGA CGTTGCTCCACCATGGACATGTGACACCAA	6074
	ACTTGGCC <u>T</u> AGGTTGGC	6075
	GCCAACCT <u>A</u> GGCCAAGT	6076
Waxy starch GBSS <i>Antirrhinum majus</i>	GGTGGAGCAACGTCTTCACCGGATACTAAAACAAACTTGGCCCAG GTTGGCCTCAGGAAC <u>T</u> AGCAATTCACTCACAATGGGTTGAGATCAA TAAACATGGTTGATAAGCTTCAAATGAGGA	6077
Gln35Term CAG-TAG	TCCTCATTTGAAGCTTATCAACCATGTTTATTGATCTCAACCCATTG TGAGTGAATTGCTAGTTCCTGAGGCCAACCTGGGCCAAGTTTGTT TTAGTATCCGGTGAAGACGTTGCTCCACC	6078

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Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID NO:
	TCAGGAAC <u>T</u> AGCAATTC	6079
	GAATTGCT <u>A</u> GTTCCTGA	6080
Waxy starch GBSS Antirrhinum majus	GGAGCAACGTCTTCACCGGATACTAAAACAAACTTGGCCCAGGTT GGCCTCAGGAACCAGTAATTCACTCACAATGGGTTGAGATCAATAA ACATGGTTGATAAGCTTCAAATGAGGAACA	6081
GIn36Term CAA-TAA	TGTTCCTCATTTGAAGCTTATCAACCATGTTTATTGATCTCAACCCA TTGTGAGTGAATTACTGGTTCCTGAGGCCAACCTGGGCCAAGTTT GTTTTAGTATCCGGTGAAGACGTTGCTCC	6082
	GGAACCAG <u>T</u> AATTCACT	6083
	AGTGAATT <u>A</u> CTGGTTCC	6084
Waxy starch GBSS Ipomoea batatas	GTGATGGCGACTATAACTGCCTCACACTTTGTTTCTCATGTCTGTG GGGGTGCCACTTCTTGAGAATCAAAAGTGGGGTTGGGTCAATTAG CCCTGAGGAGCCAAGCTGTGACTCACAATG	6085
Gly20Term GGA-TGA	CATTGTGAGTCACAGCTTGGCTCCTCAGGGCTAATTGACCCAACC CCACTTTTGATTCTCAAGAAGTGGCACCCCCACAGACATGAGAAA CAAAGTGTGAGGCAGTTATAGTCGCCATCAC	6086
	CCACTTCT <u>T</u> GAGAATCA	6087
	TGATTCTC <u>A</u> AGAAGTGG	6088
Waxy starch GBSS Ipomoea batatas	ATGGCGACTATAACTGCCTCACACTTTGTTTCTCATGTCTGTGGGG GTGCCACTTCTGGATAATCAAAAGTGGGGTTGGGTCAATTAGCCC TGAGGAGCCAAGCTGTGACTCACAATGGGT	6089
Glu21Term GAA-TAA	ACCCATTGTGAGTCACAGCTTGGCTCCTCAGGGCTAATTGACCCA ACCCCACTTTTGATTATCCAGAAGTGGCACCCCCACAGACATGAG AAACAAAGTGTGAGGCAGTTATAGTCGCCAT	6090
	CTTCTGGATAATCAAAA	6091
	TTTTGATT <u>A</u> TCCAGAAG	6092
Waxy starch GBSS Ipomoea batatas	CGACTATAACTGCCTCACACTTTGTTTCTCATGTCTGTGGGGGTGC CACTTCTGGAGAATGAAAAGTGGGGTTGGGTCAATTAGCCCTGAG GAGCCAAGCTGTGACTCACAATGGGTTGAG	6093
Ser22Term TCA-TGA	CTCAACCCATTGTGAGTCACAGCTTGGCTCCTCAGGGCTAATTGA CCCAACCCCACTTTTCATTCTCCAGAAGTGGCACCCCCACAGACA TGAGAAACAAAGTGTGAGGCAGTTATAGTCG	6094
	TGGAGAAT <u>G</u> AAAAGTGG	6095
	CCACTTTT <u>C</u> ATTCTCCA	6096

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Phenotype, Gene,		SEQID
Plant & Targeted Alteration	Altering Oligos	NO:
Waxy starch	ACTATAACTGCCTCACACTTTGTTTCTCATGTCTGTGGGGGTGCCA	6097
GBSS	CTTCTGGAGAATCATAAGTGGGGTTGGGTCAATTAGCCCTGAGGA	0031
Ipomoea batatas	GCCAAGCTGTGACTCACAATGGGTTGAGAC	
Lys23Term	GTCTCAACCCATTGTGAGTCACAGCTTGGCTCCTCAGGGCTAATT	6098
AAA-TAA	GACCCAACCCCACTTATGATTCTCCAGAAGTGGCACCCCCACAGA	3333
	CATGAGAAACAAAGTGTGAGGCAGTTATAGT	1
	GAGAATCA <u>T</u> AAGTGGGG	6099
	CCCCACTTATGATTCTC	6100
Waxy starch	CCTCACACTTTGTTTCTCATGTCTGTGGGGGTGCCACTTCTGGAGA	6101
GBSS	ATCAAAAGTGGGGT <u>A</u> GGGTCAATTAGCCCTGAGGAGCCAAGCTGT	
Ipomoea batatas	GACTCACAATGGGTTGAGACCTGTGAACAA	
Leu26Term	TTGTTCACAGGTCTCAACCCATTGTGAGTCACAGCTTGGCTCCTCA	6102
TTG-TAG	GGGCTAATTGACCCTACCCCACTTTTGATTCTCCAGAAGTGGCAC	
	CCCCACAGACATGAGAAACAAAGTGTGAGG	
	AGTGGGGT <u>A</u> GGGTCAAT	6103
	ATTGACCCTACCCCACT	6104
Waxy starch	CATCGGCGATTGTTGCTCCTTACTGCTCTCTCACAGAATGGCAAC	6105
GBSS	GGTGACGGGGTCTTA <u>G</u> GTGGTGTCGAGAAGCGCGTGCTTCAATTC	
Astragalus	CCAGGGAAGAACAGAAGCCAAAGTGAATTCA	
membranaeus	TGAATTCACTTTGGCTTCTGTTCTTCCCTGGGAATTGAAGCACGCG	6106
Tyr8Term	CTTCTCGACACCACCTAAGACCCCGTCACCGTTGCCATTCTGTGA	
TAT-TAG	GAGAGCAGTAAGGAGCAACAATCGCCGATG	
	GGGTCTTA <u>G</u> GTGGTGTC	6107
	GACACCAC <u>C</u> TAAGACCC	6108
Waxy starch	ATTGTTGCTCCTTACTGCTCTCTCACAGAATGGCAACGGTGACGG	6109
GBSS	GGTCTTATGTGGTGTAGAGAAGCGCGTGCTTCAATTCCCAGGGAA	
Astragalus	GAACAGAAGCCAAAGTGAATTCACCTCAGAA	
membranaeus	TTCTGAGGTGAATTCACTTTGGCTTCTGTTCTTCCCTGGGAATTGA	6110
Ser11Term	AGCACGCGCTTCTCTACACCACATAAGACCCCGTCACCGTTGCCA	
FCG-TAG	TTCTGTGAGAGCAGTAAGGAGCAACAAT	
	TGTGGTGT <u>A</u> GAGAAGCG	6111
	CGCTTCTCTACACCACA	6112

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Waxy starch GBSS Astragalus	TGTTGCTCCTTACTGCTCTCTCACAGAATGGCAACGGTGACGGGG TCTTATGTGGTGTCGTGAAGCGCGTGCTTCAATTCCCAGGGAAGA ACAGAAGCCAAAGTGAATTCACCTCAGAAGA	6113
membranaeus Arg12Term AGA-TGA	TCTTCTGAGGTGAATTCACTTTGGCTTCTGTTCTTCCCTGGGAATT GAAGCACGCGCTTCACGACACACATAAGACCCCGTCACCGTTGC CATTCTGTGAGAGAGCAGCAGCAACA	6114
	TGGTGTCGTGAAGCGCG	6115
	CGCGCTTC <u>A</u> CGACACCA	6116
Waxy starch GBSS Astragalus	ACTGCTCTCACAGAATGGCAACGGTGACGGGGTCTTATGTGGT GTCGAGAAGCGCGTGATTCAATTCCCAGGGAAGAACAGAAGCCAA AGTGAATTCACCTCAGAAGATAAATCTCAAT	6117
membranaeus Cys15Term TGC-TGA	ATTGAGATTTATCTTCTGAGGTGAATTCACTTTGGCTTCTGTTCTTC CCTGGGAATTGAATCACGCGCTTCTCGACACCACATAAGACCCCG TCACCGTTGCCATTCTGTGAGAGAGCAGT	6118
	AGCGCGTG <u>A</u> TTCAATTC	6119
	GAATTGAA <u>T</u> CACGCGCT	6120
Waxy starch GBSS Astragalus	CACAGAATGGCAACGGTGACGGGGTCTTATGTGGTGTCGAGAAGC GCGTGCTTCAATTCCTAGGGAAGAACAGAAGCCAAAGTGAATTCA CCTCAGAAGATAAATCTCAATAGCCAAGCAT	6121
membranaeus Gin19Term CAG-TAG	ATGCTTGGCTATTGAGATTTATCTTCTGAGGTGAATTCACTTTGGCT TCTGTTCTTCCCTAGGAATTGAAGCACGCGCTTCTCGACACCACAT AAGACCCCGTCACCGTTGCCATTCTGTG	6122
	TCAATTCC <u>T</u> AGGGAAGA	6123
	TCTTCCCT <u>A</u> GGAATTGA	6124
Waxy starch GBSS Solanum tuberosum	TGTAGCTTGGTAGATTCCCCTTTTTGTAGACCACACATCACATGGC AAGCATCACAGCTTGACACCACTTTGTGTCAAGAAGCCAAACTTCA CTAGACACCAAATCAACCTTGTCACAGAT	6125
Ser7Term TCA-TGA	ATCTGTGACAAGGTTGATTTGGTGTCTAGTGAAGTTTGGCTTCTTG ACACAAAGTGGTGTCAAGCTGTGATGCTTGCCATGTGATGTGTGG TCTACAAAAAGGGGAATCTACCAAGCTACA	6126
	CACAGCTT <u>G</u> ACACCACT	6127
	AGTGGTGT <u>C</u> AAGCTGTG	6128
Waxy starch GBSS Solanum tuberosum	TCCCCTTTTTGTAGACCACACATCACATGGCAAGCATCACAGCTTC ACACCACTTTGTGTGAAGAAGCCAAACTTCACTAGACACCAAATCA ACCTTGTCACAGATAGGACTCAGGAACCA	6129
Ser12Term TCA-TGA	TGGTTCCTGAGTCCTATCTGTGACAAGGTTGATTTGGTGTCTAGTG AAGTTTGGCTTCTT <u>C</u> ACACAAAGTGGTGTGAAGCTGTGATGCTTGC CATGTGATGTG	6130

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	CTTTGTGT <u>G</u> AAGAAGCC	6131
	GGCTTCTT <u>C</u> ACACAAAG	6132
Waxy starch GBSS Solanum tuberosum	CCCTTTTGTAGACCACACATCACATGGCAAGCATCACAGCTTCAC ACCACTTTGTGTCATGAAGCCAAACTTCACTAGACACCAAATCAAC CTTGTCACAGATAGGACTCAGGAACCATA	6133
Arg13Term AGA-TGA	TATGGTTCCTGAGTCCTATCTGTGACAAGGTTGATTTGGTGTCTAG TGAAGTTTGGCTTCATGACACAAAGTGGTGTGAAGCTGTGATGCTT GCCATGTGATGTG	6134
	TTGTGTCA <u>T</u> GAAGCCAA	6135
	TTGGCTTC <u>A</u> TGACACAA	6136
Waxy starch GBSS Solanum tuberosum	TTGTAGACCACACATCACATGGCAAGCATCACAGCTTCACACCACT TTGTGTCAAGAAGCTAAACTTCACTAGACACCAAATCAACCTTGTC ACAGATAGGACTCAGGAACCATACTCTGA	6137
GIn15Term CAA-TAA	TCAGAGTATGGTTCCTGAGTCCTATCTGTGACAAGGTTGATTTGGT GTCTAGTGAAGTTTAGCTTCTTGACACAAAGTGGTGTGAAGCTGTG ATGCTTGCCATGTGATGTG	6138
	CAAGAAGC <u>T</u> AAACTTCA	6139
	TGAAGTTT <u>A</u> GCTTCTTG	6140
Waxy starch GBSS Solanum tuberosum	CCACACATCACATGGCAAGCATCACAGCTTCACACCACTTTGTGTC AAGAAGCCAAACTTGACTAGACACCAAATCAACCTTGTCACAGATA GGACTCAGGAACCATACTCTGACTCACAA	6141
Ser17Term TCA-TGA	TTGTGAGTCAGAGTATGGTTCCTGAGTCCTATCTGTGACAAGGTTG ATTTGGTGTCTAGTCAAGTTTGGCTTCTTGACACAAAGTGGTGTGA AGCTGTGATGCTTGCCATGTGATGTG	6142
	CCAAACTT <u>G</u> ACTAGACA	6143
	TGTCTAGT <u>C</u> AAGTTTGG	6144
Waxy starch GBSS Pisum sativum	GTCGATCACTCTTCTCCACCGCCGAAACAGATTTTGACACAAAAA TGGCAACAATAACG <u>T</u> GATCTTCAATGCCGACGAGAACCGCGTGCT TCAATTACCAAGGAAGATCAGCAGAGTCTA	6145
Gly6Term GGA-TGA	TAGACTCTGCTGATCTTCCTTGGTAATTGAAGCACGCGGTTCTCGT CGGCATTGAAGATCACGTTATTGTTGCCATTTTTGTGTCAAAATCT GTTTCGGCGGTGAGAGAAGAGTGATCGAC	6146
	CAATAACG <u>T</u> GATCTTCA	6147
	TGAAGATC <u>A</u> CGTTATTG	6148

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Waxy starch GBSS Pisum sativum	ACTCTTCTCTCACCGCCGAAACAGATTTTGACACAAAAATGGCAAC AATAACGGGATCTTGAATGCCGACGAGAACCGCGTGCTTCAATTA CCAAGGAAGATCAGCAGAGTCTAAACTGAA	6149
Ser8Term TCA-TGA	TTCAGTTTAGACTCTGCTGATCTTCCTTGGTAATTGAAGCACGCGG TTCTCGTCGGCATTCAAGATCCCGTTATTGTTGCCATTTTTGTGTC AAAATCTGTTTCGGCGGTGAGAGAAGAGT	6150
	GGGATCTT <u>G</u> AATGCCGA	6151
	TCGGCATT <u>C</u> AAGATCCC	6152
Waxy starch GBSS Pisum sativum	ACCGCCGAAACAGATTTTGACACAAAAATGGCAACAATAACGGGAT CTTCAATGCCGACG <u>T</u> GAACCGCGTGCTTCAATTACCAAGGAAGAT CAGCAGAGTCTAAACTGAATTTGCCTCAGA	6153
Arg12Term AGA-TGA	TCTGAGGCAAATTCAGTTTAGACTCTGCTGATCTTCCTTGGTAATT GAAGCACGCGGTTCACGTCGGCATTGAAGATCCCGTTATTGTTGC CATTTTTGTGTCAAAATCTGTTTCGGCGGT	6154
	TGCCGACG <u>T</u> GAACCGCG	6155
	CGCGGTTC <u>A</u> CGTCGGCA	6156
Waxy starch GBSS Pisum sativum	AGATTTTGACACAAAAATGGCAACAATAACGGGATCTTCAATGCCG ACGAGAACCGCGTGATTCAATTACCAAGGAAGATCAGCAGAGTCT AAACTGAATTTGCCTCAGATACACTTCAAT	6157
Cys15Term TGC-TGA	ATTGAAGTGTATCTGAGGCAAATTCAGTTTAGACTCTGCTGATCTT CCTTGGTAATTGAATCACGCGGTTCTCGTCGGCATTGAAGATCCC GTTATTGTTGCCATTTTTGTGTCAAAATCT	6158
	ACCGCGTGATTCAATTA .	6159
	TAATTGAA <u>T</u> CACGCGGT	6160
Waxy starch GBSS Pisum sativum	CACAAAAATGGCAACAATAACGGGATCTTCAATGCCGACGAGAAC CGCGTGCTTCAATTAGCAAGGAAGATCAGCAGAGTCTAAACTGAAT TTGCCTCAGATACACTTCAATAACAACCAA	6161
Tyr18Term TAC-TAG	TTGGTTGTTATTGAAGTGTATCTGAGGCAAATTCAGTTTAGACTCTG CTGATCTTCCTTGCTAATTGAAGCACGCGGTTCTCGTCGGCATTGA AGATCCCGTTATTGTTGCCATTTTTGTG	6162
	TTCAATTA <u>G</u> CAAGGAAG	6163
	CTTCCTTG <u>C</u> TAATTGAA	6164
Waxy starch GBSS Manihot esculenta	TCTACACCGGAGAGAGCACCATGGCAACTGTAATAGCTGCACATT TCGTTTCCAGGAGCTGACACTTGAGCATCCATGCATTAGAGACTAA GGCTAATAATTTGTCTCACACTGGACCCTG	6165
Ser14Term TCA-TGA	CAGGGTCCAGTGTGAGACAAATTATTAGCCTTAGTCTCTAATGCAT GGATGCTCAAGTGTCAGCTCCTGGAAACGAAATGTGCAGCTATTA CAGTTGCCATGGTGCTCTCCCGGTGTAGA	6166

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
	CAGGAGCTGACACTTGA	6167
	TCAAGTGT <u>C</u> AGCTCCTG	6168
Waxy starch GBSS Manihot esculenta	CCGGAGAGAGCACCATGGCAACTGTAATAGCTGCACATTTCGTTT CCAGGAGCTCACACTAGAGACTAAGGCTA ATAATTTGTCTCACACTGGACCCTGGACCCA	6169
Leu16Term TTG-TAG	TGGGTCCAGGGTCCAGTGTGAGACAAATTATTAGCCTTAGTCTCTA ATGCATGGATGCTCTAGTGTGAGCTCCTGGAAACGAAATGTGCAG CTATTACAGTTGCCATGGTGCTCTCTCCGG	6170
	CTCACACT <u>A</u> GAGCATCC	6171
	GGATGCTC <u>T</u> AGTGTGAG	6172
Waxy starch GBSS Manihot esculenta	TGGCAACTGTAATAGCTGCACATTTCGTTTCCAGGAGCTCACACTT GAGCATCCATGCATGAGAGACTAAGGCTAATAATTTGTCTCACACT GGACCCTGGACCCAAACTATCACTCCCAA	6173
Leu21Term TTA-TGA	TTGGGAGTGATAGTTTGGGTCCAGGGTCCAGTGTGAGACAAATTA TTAGCCTTAGTCTCCATGCATGGATGCTCAAGTGTGAGCTCCTGG AAACGAAATGTGCAGCTATTACAGTTGCCA	6174
	CCATGCAT <u>G</u> AGAGACTA	6175
	TAGTCTCT <u>C</u> ATGCATGG	6176
Waxy starch GBSS Manihot esculenta	GCAACTGTAATAGCTGCACATTTCGTTTCCAGGAGCTCACACTTGA GCATCCATGCATTATAGACTAAGGCTAATAATTTGTCTCACACTGG ACCCTGGACCCAAACTATCACTCCCAATG	6177
Glu22Term GAG-TAG	CATTGGGAGTGATAGTTTGGGTCCAGGGTCCAGTGTGAGACAAAT TATTAGCCTTAGTCTATAATGCATGGATGCTCAAGTGTGAGCTCCT GGAAACGAAATGTGCAGCTATTACAGTTGC	6178
	ATGCATTA <u>T</u> AGACTAAG	6179
	CTTAGTCT <u>A</u> TAATGCAT	6180
Waxy starch GBSS Manihot esculenta	GTAATAGCTGCACATTTCGTTTCCAGGAGCTCACACTTGAGCATCC ATGCATTAGAGACTTAGGCTAATAATTTGTCTCACACTGGACCCTG GACCCAAACTATCACTCCCAATGGTTTAA	6181
Lys24Term AAG-TAG	TTAAACCATTGGGAGTGATAGTTTGGGTCCAGGGTCCAGTGTGAG ACAAATTATTAGCCTAAGTCTCTAATGCATGGATGCTCAAGTGTGA GCTCCTGGAAACGAAATGTGCAGCTATTAC	6182
	TAGAGACT <u>T</u> AGGCTAAT	6183
	ATTAGCCT <u>A</u> AGTCTCTA	6184

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Waxy starch GBSS Phaseolus vulgaris	ACAACTCCTCCGTCACCGGTATAAGCATGGCAACGGTATCGATGG CATCGTGCGTGGCGTG	6185
Ser12Term TCA-TGA	CGGTTCAGGCTCATCTGACCCGAAGATTTCACTTTTGTCTCTGTAC TCCACGCGCCTTTTCACGCCACGC	6186
	CGTGGCGT <u>G</u> AAAAGGCG	6187
	CGCCTTTT <u>C</u> ACGCCACG	6188
Waxy starch GBSS Phaseolus vulgaris	CACCGGTATAAGCATGGCAACGGTATCGATGGCATCGTGCCTGGC GTCAAAAGGCGCGTGAAGTACAGAGACAAAAGTGAAATCTTCGGG TCAGATGAGCCTGAACCGTCATGAATTGAAA	6189
Trp16Term TGG-TGA	TTTCAATTCATGACGGTTCAGGCTCATCTGACCCGAAGATTTCACT TTTGTCTCTGTACTTCACGCGCCTTTTGACGCCACGCACG	6190
	GGCGCGTG <u>A</u> AGTACAGA	6191
	TCTGTACTTCACGCGCC	6192
Waxy starch GBSS Phaseolus vulgaris	ATAAGCATGGCAACGGTATCGATGGCATCGTGCGTGGCGTCAAAA GGCGCGTGGAGTACA <u>T</u> AGACAAAAGTGAAATCTTCGGGTCAGATG AGCCTGAACCGTCATGAATTGAAATACGATG	6193
Glu19Term GAG-TAG	CATCGTATTTCAATTCATGACGGTTCAGGCTCATCTGACCCGAAGA TTTCACTTTTGTCTATGTACTCCACGCGCCTTTTGACGCCACGCAC GATGCCATCGATACCGTTGCCATGCTTAT	6194
	GGAGTACA <u>T</u> AGACAAAA	6195
	TTTTGTCTATGTACTCC	6196
Waxy starch GBSS Phaseolus vulgaris	ATGGCAACGGTATCGATGGCATCGTGCGTGGCGTCAAAAGGCGC GTGGAGTACAGAGACATAAGTGAAATCTTCGGGTCAGATGAGCCT GAACCGTCATGAATTGAAATACGATGGGTTGA	6197
Lys21Term AAA-TAA	TCAACCCATCGTATTTCAATTCATGACGGTTCAGGCTCATCTGACC CGAAGATTTCACTTATGTCTCTGTACTCCACGCGCCTTTTGACGCC ACGCACGATGCCATCGATACCGTTGCCAT	6198
	CAGAGACA <u>T</u> AAGTGAAA	6199
	TTTCACTT <u>A</u> TGTCTCTG	6200
Waxy starch GBSS Phaseolus vulgaris	ACGGTATCGATGGCATCGTGCGTGGCGTCAAAAGGCGCGTGGAG TACAGAGACAAAAGTGTAATCTTCGGGTCAGATGAGCCTGAACCG TCATGAATTGAAATACGATGGGTTGAGATCTC	6201
Lys23Term AAA-TAA	GAGATCTCAACCCATCGTATTTCAATTCATGACGGTTCAGGCTCAT CTGACCCGAAGATTACACTTTTGTCTCTGTACTCCACGCGCCTTTT GACGCCACGCACGATGCCATCGATACCGT	6202

Phenotype, Gene, Plant & Targeted Alteration	Aftering Oligos	SEQ II
	CAAAAGTG <u>T</u> AATCTTCG	6203
	CGAAGATT <u>A</u> CACTITTG	6204
Waxy starch GBSS Triticum aestivum	GCGCCTAGCTCGAAAAGGTCGTCATTGAGAGGCTGCACCAATGG GTTCCATTCCTAATTAGTGTTCTTATCAAACAAACAGTGTTGGTTCA CTGAAACTGTCGCCTCACATCCAATTCCAG	1
TAT-TAG	CTGGAATTGGATGTGAGGCGACAGTTTCAGTGAACCAACACTGTTT GTTTGATAAGAACACTAATTAGGAATGGAACCCATTGGTGCAGCCT CTCAATGACGACCTTTTCGAGCTAGGCGC CCTAATTAGTGTTCTTA	
	TAAGAACACTAATTAGG	6207
Waxy starch GBSS Triticum aestivum	CCTAGCTCGAAAAGGTCGTCATTGAGAGGCTGCACCAATGGGTTC CATTCCTAATTATTGATCTTATCAAACAACAGTGTTGGTTCACTGA AACTGTCGCCTCACATCCAATTCCAGCAA	6208
Cys8Term TGT-TGA	TTGCTGGAATTGGATGTGAGGCGACAGTTTCAGTGAACCAACACT GTTTGTTTGATAAGA <u>T</u> CAATAATTAGGAATGGAACCCATTGGTGCA GCCTCTCAATGACGACCTTTTCGAGCTAGG	6210
•	AATTATTG <u>A</u> TCTTATCA TGATAAGATCAATAATT	6211
Waxy starch GBSS Triticum aestivum	TCGAAAAGGTCGTCATTGAGAGGCTGCACCAATGGGTTCCATTCC TAATTATTGTTCTTAGCAAACAAACAGTGTTGGTTCACTGAAACTGT CGCCTCACATCCAATTCCAGCAATCTTGT	6212 6213
Tyr10Term TAT-TAG	ACAAGATTGCTGGAATTGGATGTGAGGCGACAGTTTCAGTGAACC AACACTGTTTGTTTGCTAAGAACAATAATTAGGAATGGAACCCATT GGTGCAGCCTCTCAATGACGACCTTTTCGA	6214
	TGTTCTTAGCAAACAAA TTTGTTTGCTAAGAACA	6215
Waxy starch GBSS Triticum aestivum	CGAAAAGGTCGTCATTGAGAGGCTGCACCAATGGGTTCCATTCCT AATTATTGTTCTTATTAAACAAACAGTGTTGGTTCACTGAAACTGTC GCCTCACATCCAATTCCAGCAATCTTGTA	6216 6217
GIn11Term CAA-TAA	TACAAGATTGCTGGAATTGGATGTGAGGCGACAGTTTCAGTGAAC CAACACTGTTTGTTTAATAAGAACAATAATTAGGAATGGAACCCATT GGTGCAGCCTCTCAATGACGACCTTTTCG	6218
	GTTCTTATTAAACAAC	6219
	GTTTGTTT <u>A</u> ATAAGAAC	6220

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Phenotype, Gene, Plant & Targeted Alteration	Attering Oligos	SEQID NO:
Waxy starch GBSS Triticum aestivum	AGGCTGCACCAATGGGTTCCATTCCTAATTATTGTTCTTATCAAACA AACAGTGTTGGTTGACTGAAACTGTCGCCTCACATCCAATTCCAGC AATCTTGTAACAATGAAGTTATGTTCCT	6221
Ser17Term TCA-TGA	AGGAACATAACTTCATTGTTACAAGATTGCTGGAATTGGATGTGAG GCGACAGTTTCAGTCAACCAACACTGTTTGTTTGATAAGAACAATA ATTAGGAATGGAACCCATTGGTGCAGCCT	6222
	TGTTGGTT <u>G</u> ACTGAAAC	6223
	GTTTCAGT <u>C</u> AACCAACA	6224
Waxy starch GBSS Triticum aestivum	CAGCTCGCCACCTCCGGCACCGTCCTCGGCATCACCGACAGGTT CCGGCGTGCAGGTTTCTAGGGCGTGAGGCCCGGAGCCCGGCG GATGCGGCTCTCGGCATGAGGACCGTCGGAGCTA	6225
GIn28Term CAG-TAG	TAGCTCCGACGGTCCTCATGCCGAGAGCCGCATCCGCCGGGCTCCGGGGCCTCACGCCCTAGAAACCTGCACGCCGGAACCTGTCGGTGATGCCGAGGAGGTGCCGGAGGTGGCGAGCTG	6226
	CAGGTTTC <u>T</u> AGGGCGTG	6227
	CACGCCCT <u>A</u> GAAACCTG	6228
Waxy starch GBSS Triticum aestivum	GGTTTCCAGGGCGTGAGGCCCCGGAGCCCGGCGGATGCGGCTC TCGGCATGAGGACCGTCTGAGCTAGCGCCGCCCCAACGCAAAGC CGGAAAGCGCACCGCGGGACCCGGCGGTGCCTCT	6229
Gly46Term GGA-TGA	AGAGGCACCGCCGGGTCCCGCGGTGCGCTTTCCGGCTTTGCGTT GGGGCGGCGCTAGCTCAGACCGTCCTCATGCCGAGAGCCGCATC CGCCGGGCTCCGGGGCCTCACGCCCTGGAAACC	6230
	GGACCGTC <u>T</u> GAGCTAGC	6231
	GCTAGCTCAGACGGTCC	6232
Waxy starch GBSS Triticum aestivum	CGGAGCCCGGCGATGCGGCTCTCGGCATGAGGACCGTCGGAG CTAGCGCCCCCAACGTAAAGCCGGAAAGCGCACCGCGGGACC CGGCGGTGCCTCTCCATGGTGGTGCGCGCCACCG	6233
GIn53Term CAA-TAA	CGGTGGCGCACCACCATGGAGAGGCACCGCCGGGTCCCGCG GTGCGCTTTCCGGCTTTACGTTGGGGCGCGCTAGCTCCGACGG TCCTCATGCCGAGAGCCGCATCCGCCGGGCTCCG	6234
	CCCCAACG <u>T</u> AAAGCCGG	6235
	CCGGCTTTACGTTGGGG	6236
Waxy starch GBSS Triticum aestivum	GCGGATGCGGCTCTCGGCATGAGGACCGTCGGAGCTAGCGCCG CCCCAACGCAAAGCCGGTAAGCGCACCGCGGGACCCGGCGGTG CCTCTCCATGGTGGTGCGCGCCACCGGCAGCGGCG	6237
Lys56Term AAA-TAA	CGCCGCTGCCGGTGGCGCGCACCACCATGGAGAGGCACCGCCGGGTCCCGCGGTGCGCTTACCGGCTTTGCGTTGGGGCGCGCTAGCTCCGACGGTCCTCATGCCGAGAGCCGCATCCGC	6238

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	AAAGCCGGTAAGCGCAC .	6239
	GTGCGCTT <u>A</u> CCGGCTTT	6240
Waxy starch GBSS Triticum aestivum	CTCTCCATGGTGGTGCGCGCCACCGGCAGCGGCGCATGAACCT CGTGTTCGTCGGCGCCTAGATGGCGCCCTGGAGCAAGACCGGCG GCCTCGGCGACGTCCTCGGGGGCCTCCCCCAG	6241
Glu85Term GAG-TAG	CTGGGGGAGGCCCCGAGGACGTCGCCGAGGCCGCCGGTCTT GCTCCAGGGCGCCATCTAGGCGCCGACGAACACGAGGTTCATGC CGCCGCTGCCGGTGGCGCGCACCACCATGGAGAG	6242
	TCGGCGCCTAGATGGCG	6243
	CGCCATCTAGGCGCCGA	6244
Waxy starch GBSS Triticum aestivum	GTCGTCTCGCTGCAGGTAGCCACACCCTGCGCGCGCGATGGC GGCTCTGGTCACGTCGTAGCTCGCCACCTCCGGCACCGTCCTCG GCATCACCGACAGGTTCCGGCGTGCAGGTTTTC	6245
Gin8Term CAG-TAG	GAAAACCTGCACGCCGGAACCTGTCGGTGATGCCGAGGACGGTG CCGGAGGTGGCGAGCTACGACGTGACCAGAGCCGCCATCGCGC GCGCAGGGTGTGGCTACCTGCAGCGAGAGACGAC	6246
	TCACGTCG <u>T</u> AGCTCGCC	6247
	GGCGAGCT <u>A</u> CGACGTGA	6248
Waxy starch GBSS Triticum aestivum	CAGCTCGCCACCTCCGGCACCGGCACAGGTT CCGGCGTGCAGGTTTTTAGGGTGTGAGGCCCCGGAGCCCGGCAG ATGCGCCGCTCGGCATGAGGACTACCGGAGCGA	6249
Gln28Term CAG-TAG	TCGCTCCGGTAGTCCTCATGCCGAGCGGCGCATCTGCCGGGCTC CGGGGCCTCACACCCTAAAAACCTGCACGCCGGAACCTGTCGGT GATGCCGAGGACGGTGCCGGAGGTGGCGAGCTG	6250
	CAGGTTTTTAGGGTGTG	6251
	CACACCCT <u>A</u> AAAACCTG	6252
Waxy starch GBSS Triticum aestivum	CCCCGGAGCCCGGCAGATGCGCCGCTCGGCATGAGGACTACCG GAGCGAGCGCCCCCGTAGCAACAAAGCCGGAAAGCGCACCG CGGGACCCGGCGGTGCCTCTCCATGGTGGTGCGCG	6253
Lys52Term AAG-TAG	CGCGCACCACGTGGAGAGGCACCGCCGGGTCCCCGCGTGCGC TTTCCGGCTTTGTTGCTACGGGGCGCGCCTCCGCTAGTCC TCATGCCGAGCGCGCATCTGCCGGGCTCCGGGG	6254
	CCGCCCGTAGCAACAA	6255
	TTGTTGCTACGGGGCGG	6256

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Waxy starch GBSS Triticum aestivum	CGGAGCCCGGCAGATGCGCCGCTCGGCATGAGGACTACCGGAG CGAGCGCCGCCCGAAGTAACAAAGCCGGAAAGCGCACCGCGG GACCCGGCGGTGCCTCTCCATGGTGCTGCGCCCCA	6257
GIn53Term CAA-TAA	TGGCGCGCACCACCATGGAGAGGCACCGCCGGGTCCCGCGGTG CGCTTTCCGGCTTTGTTACTTCGGGGCGCGCGCTCGCTCCGGTAG TCCTCATGCCGAGCGCGCATCTGCCGGGCTCCG	6258
	CCCCGAAG <u>T</u> AACAAAGC	6259
Waxy starch GBSS	GCTTTGTTACTTCGGGG  AGCCCGGCAGATGCGCCGCTCGGCATGAGGACTACCGGAGCGA GCGCCGCCCCGAAGCAATAAAGCCGGAAAGCGCACCGCGGAC	6260 6261
Triticum aestivum Gln54Term CAA-TAA	CCGGCGTGCCTCTCCATGGTGGTGCGCGCCACGG CCGTGGCGCACCACCATGGAGAGGCACCGCCGGGTCCCGCG GTGCGCTTTCCGGCTTTATTGCTTCGGGGCGCGCTCGCTC	6262
,	CGAAGCAATAAAGCCGG	6263
	CCGGCTTTATTGCTTCG	6264
Waxy starch GBSS Triticum durum	CAGCTCGCCACCTCCGGCACCGTCCTCGGCATCACCGACAGGTT CCGGCGTGCAGGTTTCTAGGGCGTGAGGCCCCGGAACCCGGCG GATGCGGCCCTCGTCATGAGGACTATCGGAGCGA	6265
GIn28Term CAG-TAG	TCGCTCCGATAGTCCTCATGACGAGGGCCGCATCCGCCGGGTTC CGGGGCCTCACGCCCTAGAAACCTGCACGCCGGAACCTGTCGGT GATGCCGAGGACGGTGCCGGAGGTGGCGAGCTG	6266
	CAGGTTTC <u>T</u> AGGGCGTG	6267
	CACGCCCT <u>A</u> GAAACCTG	6268
Waxy starch GBSS Triticum durum	CCCCGGAACCCGGCGGATGCGGCCCTCGTCATGAGGACTATCGG AGCGAGCGCCCCCGTAGCAAAGCCGGAAAGCGCACCGCGGG AGCCGGCGGTGCCTCTCCATGGTGGTGCGCGCCA	6269
Lys52Term AAG-TAG	TGGCGCGCACCACGATGGAGAGGCACCGCCGGCTCCCGCGGTG CGCTTTCCGGCTTTGCTACGGGGCGCGCTCCGCTC	6270
	CCGCCCG <u>T</u> AGCAAAGC	6271
	GCTTTGCTACGGGGCGG	6272
Waxy starch GBSS Triticum durum	CGGAACCCGGCGATGCGGCCCTCGTCATGAGGACTATCGGAGC GAGCGCCGCCCGAAG <u>T</u> AAAGCCGGAAAGCGCACCGCGGAGC CGGCGGTGCCTCTCCATGGTGGTGCGCGCCACGG	6273
GIn53Term CAA-TAA	CCGTGGCGCGCACCACGATGGAGAGGCACCGCCGGCTCCCGCG GTGCGCTTTCCGGCTTTACTTCGGGGCGGCGCTCGCTCCGATAG TCCTCATGACGAGGGCCGCATCCGCCGGGTTCCG	6274

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	CCCCGAAGTAAAGCCGG	6275
	CCGGCTTTACTTCGGGG	6276
Waxy starch	GCGGATGCGGCCCTCGTCATGAGGACTATCGGAGCGAGCG	6277
GBSS	CCCGAAGCAAAGCCGGTAAGCGCACCGCGGGAGCCGGCGGTGC	ļ
Triticum durum	CTCTCCATGGTGCTGCGCGCCACGGGCAGCGGCG	
Lys56Term AAA-TAA	CGCCGCTGCCGTGGCGCGCACCACCATGGAGAGGCACCGCCG	6278
AAA-TAA	GCTCCCGCGGTGCGCTTACCGGCTTTGCTTCGGGGCGCGCTCG	
	CTCCGATAGTCCTCATGACGAGGGCCGCATCCGC	
ĺ	AAAGCCGG <u>T</u> AAGCGCAC	6279
	GTGCGCTT <u>A</u> CCGGCTTT	6280
Waxy starch	TATCGGAGCGAGCGCCCCCGAAGCAAGCCGGAAAGCGCACC	6281
GBSS	GCGGGAGCCGGCGGTGACTCTCCATGGTGGTGCGCGCCACGGG	
Triticum durum	CAGCGGCGCATGAACCTCGTGTTCGTCGGCGCC	
Cys64Term	GGCGCCGACGAACACGAGGTTCATGCCGCCGCTGCCCGTGGCG	6282
TGC-TGA	CGCACCACCATGGAGAGTCACCGCCGGCTCCCGCGGTGCGCTTT	
	CCGGCTTTGCTTCGGGGCGCGCTCGCTCCGATA	
	CGGCGGTG <u>A</u> CTCTCCAT	6283
	ATGGAGAG <u>T</u> CACCGCCG	6284
Waxy starch	CAGCTCGCCACCTCCGGCACCGTCCTCGGCATCACCGACAGGTT	6285
GBSS	CCGCCGTGCAGGTTTT <u>T</u> AGGGTGTGAGGCCCGGAGCCCGGCAG	
Triticum turgidum	ATGCGCCGCTCGGCATGAGGACTACCGGAGCGA	
Gln28Term	TCGCTCCGGTAGTCCTCATGCCGAGCGGCGCATCTGCCGGGCTC	6286
CAG-TAG	CGGGGCCTCACACCCTAAAAACCTGCACGCCGGAACCTGTCGGT	
	GATGCCGAGGTGCCGGAGGTGGCGAGCTG	
	CAGGTTTT <u>T</u> AGGGTGTG	6287
	CACACCCT <u>A</u> AAAACCTG	6288
Waxy starch	CCCCGGAGCCCGCAGATGCGCCGCTCGGCATGAGGACTACCG	6289
GBSS	GAGCGAGCGCCCCCGTAGCAACAAAGCCGGAAAGCGCACCG	
Triticum turgidum	CGGGACCCGGCGTGCCTCTCCATGGTGGTGCGCG	
Lys52Term	CGCGCACCACCATGGAGAGGCACCGCCGGGTCCCGCGGTGCGC	6290
AAG-TAG	TTTCCGGCTTTGTTGCTACGGGGGGGGGGCGCTCGCTCCGGTAGTCC	
***	TCATGCCGAGCGCGCATCTGCCGGGGCTCCGGGG	·
	CCGCCCGTAGCAACAA	6291
	TTGTTGCTACGGGGCGG	6292

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Waxy starch GBSS Triticum turgidum	CGGAGCCCGCAGATGCGCCGCTCGGCATGAGGACTACCGGAG CGAGCGCCGCCCGAAGTAACAAAGCCGGAAAGCGCACCGCGG GACCCGGCGGTGCCTCTCCATGGTGGTGCGCGCCA	6293
GIn53Term CAA-TAA	TGGCGCGCACCACCATGGAGAGGCACCGCCGGGTCCCGCGGTGCGCTTTCCGGCTTTGTTACTTCGGGGCGCGCGC	6294
	CCCCGAAGTAACAAAGC	6295
	GCTTGTT <u>A</u> CTTCGGGG	6296
Waxy starch GBSS Triticum turgidum	AGCCCGGCAGATGCGCCGCTCGGCATGAGGACTACCGGAGCGA GCGCCGCCCGAAGCAATAAAGCCGGAAAGCGCACCGCGGGAC CCGGCGGTGCCTCTCCATGGTGGTGCGCGCCACGG	6297
Gin54Term CAA-TAA	CCGTGGCGCACCACCATGGAGAGGCACCGCCGGGTCCCGCG GTGCGCTTTCCGGCTTTATTGCTTCGGGGCGCGCGCTCCCGG TAGTCCTCATGCCGAGCGCGCATCTGCCGGGCT	6298
	CGAAGCAA <u>T</u> AAAGCCGG	6299
	CCGGCTTTATTGCTTCG	6300
Waxy starch GBSS Triticum turgidum	GATGCGCCGCTCGGCATGAGGACTACCGGAGCGAGCGCCCCCCGAAGCAACAAAGCCGGTAAGCGCACCGCGGACCCGGCGTGCCTCTCCATGGTGGTGCGCGCCACGGGCAGCGCCG	6301
Lys57Term AAA-TAA	CGGCGCTGCCGTGGCGCGCACCACCATGGAGAGGCACCGCCG GGTCCCGCGTGCGCTTACCGGCTTTGTTGCTTCGGGGCGCGCC TCGCTCCGGTAGTCCTCATGCCGAGCGCGCGCATC	6302
	AAAGCCGGTAAGCGCAC	6303
	GTGCGCTT <u>A</u> CCGGCTTT	6304
Waxy starch GBSS Aegilops speltoides	CAGCTCGCCACCTCCGCCACCGTCCTCGGCATCACCGACAGGTT CCGCCATGCAGGTTTCTAGGGCGTGAGGCCCCGGAGCCCGGCAG ATGCGCCGCTCGGCATGAGGACTGTCGGAGCGA	6305
Gin28Term CAG-TAG	TCGCTCCGACAGTCCTCATGCCGAGCGGCGCATCTGCCGGGCTC CGGGGCCTCACGCCCTAGAAACCTGCATGGCGGAACCTGTCGGT GATGCCGAGGACGGTGGCGGAGCTG	6306
	CAGGTTTCTAGGGCGTG	6307
	CACGCCCT <u>A</u> GAAACCTG	6308
Waxy starch GBSS Aegilops speltoides	GGTTTCCAGGGCGTGAGGCCCCGGAGCCCGGCAGATGCGCCGC TCGGCATGAGGACTGTCTGAGCGAGCGCCGCCCCGAAGCAACAA AGCCGGAAAGCGCACCGCGGGGACCCGGCGGTGCC	6309
Gly46Term GGA-TGA	GGCACCGCCGGGTCCCGCGGTGCGCTTTCCGGCTTTGTTGCTTC GGGGCGGCGCCTCAGACAGTCCTCATGCCGAGCGGCGCAT CTGCCGGGCTCCGGGGCCTCACGCCCTGGAAACC	6310

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	GGACTGTC <u>T</u> GAGCGAGC	6311
	GCTCGCTC <u>A</u> GACAGTCC	6312
Waxy starch GBSS Aegilops speltoides	CCCCGGAGCCCGGCAGATGCGCCGCTCGGCATGAGGACTGTCG GAGCGAGCGCCCCCGTAGCAACAAGCCGGAAAGCGCACCG CGGGACCCGGCGGTGCCTCTCGATGGTGGTGCGCG	6313
Lys52Term AAG-TAG	CGCGCACCACCATCGAGAGGCACCGCCGGGTCCCGCGGTGCGC TTTCCGGCTTTGTTGCTACGGGGCGCGCGCTCCGACAGTCC TCATGCCGAGCGGCGCATCTGCCGGGCTCCGGGG	6314
	CCGCCCGTAGCAACAA	6315
	TTGTTGCTACGGGGCGG	6316
Waxy starch GBSS Aegilops speltoides	CGGAGCCCGCAGATGCGCCGCTCGGCATGAGGACTGTCGGAG CGAGCGCCCCCGAAGTAACAAAGCCGGAAAGCGCACCGCGG GACCCGGCGGTGCCTCTCGATGGTGGTGCGCGCCA	6317
GIn53Term CAA-TAA	TGGCGCGCACCACCATCGAGAGGCACCGCCGGGTCCCGCGGTG CGCTTTCCGGCTTTGTTACTTCGGGGCGCGCCTCCGACAG TCCTCATGCCGAGCGCGCATCTGCCGGGCTCCG	6318
]	CCCCGAAGTAACAAAGC	6319
	GCTTTGTT <u>A</u> CTTCGGGG	6320
Waxy starch GBSS Aegilops speltoides	AGCCCGCAGATGCGCCGCTCGGCATGAGGACTGTCGGAGCGA GCGCCGCCCGAAGCAATAAAGCCGGAAAGCGCACCGCGGGAC CCGGCGGTGCCTCTCGATGGTGGTGCGCGCCACCG	6321
GIn54Term CAA-TAA	CGGTGGCGCACCACCATCGAGAGGCACCGCCGGGTCCCGCGGTGCGCTTTATTGCTTCGGGGCGGCGCTCGCT	6322
	CGAAGCAA <u>T</u> AAAGCCGG	6323
	CCGCTTTATTGCTTCG	6324
Waxy starch GBSS Oryza glaberrima	AGTGCAGAGATCTTCCACAGCAACAGCTAGACAACCACCATGTCG GCTCTCACCACGTCCTAGCTCGCCACCTCGGCCACCGGCTTCGG CATCGCTGACAGGTCGCCGCCGTCGTCGCTGC	6325
GIn8Term CAG-TAG	GCAGCGACGACGCGCCGACCTGTCAGCGATGCCGAAGCCGGT GGCCGAGGTGGCGAGCTAGGAGCCGACATGGTG GTTGTCTAGCTGTTGCTGTGGAAGATCTCTGCACT	6326
	CCACGTCCTAGCTCGCC	6327
	GGCGAGCT <u>A</u> GGACGTGG	6328

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Phenotype, Gene, Plant & Targeted Alteration	Attering Oligos	SEQID No:
Waxy starch GBSS Oryza glaberrima	TCCACAGCAACAGCTAGACAACCACCATGTCGGCTCTCACCACGT CCCAGCTCGCCACCTAGGCCACCGGCTTCGGCATCGCTGACAGG TCGGCGCCGTCGTCGCTGCTCCGCCACGGGTT	6329
Ser12Term TCG-TAG	AACCCGTGGCGGAGCAGCGACGACGCGACCTGTCAGCGAT GCCGAAGCCGGTGGCCTAGGTGGCGAGCTGGGACGTGGTGAGA GCCGACATGGTGGTTGTCTAGCTGTTGCTGTGGA	6330
	CGCCACCTAGGCCACCG	6331
-	CGGTGGCCTAGGTGGCG	6332
Waxy starch GBSS Onyza glabernima	CGGCTCTCACCACGTCCCAGCTCGCCACCTCGGCCACCGGCTTC GGCATCGCTGACAGGTAGGCGCCGTCGTCGCTCGCCACGG GTTCCAGGGCCTCAAGCCCCGCAGCCCCGCGG	6333
Ser22Term TCG-TAG	CCGGCGGGCTGCGGGCTTGAGGCCCTGGAACCCGTGGCGA GCAGCGACGACGCGCCTACCTGTCAGCGATGCCGAAGCCGGTG GCCGAGGTGGCGAGCTGGGACGTGAGAGCCG	6334
	TGACAGGT <u>A</u> GGCGCCGT	6335
	ACGGCGCC <u>T</u> ACCTGTCA	6336
Waxy starch GBSS Oryza glaberrima	CCACGTCCCAGCTCGCCACCTCGGCCACCGGCTTCGCATCGCT GACAGGTCGCGCCGTAGTCGCTGCTCCGCCACGGGTTCCAGG GCCTCAAGCCCCGCAGCCCCGCCGCGCGCGACGC	6337
Ser25Term TCG-TAG	GCGTCGCCGCCGGGGGCTGCGGGGCTTGAGGCCCTGGAACC CGTGGCGAGCAGCGACTACGGCGCCGACCTGTCAGCGATGCC GAAGCCGGTGGCCGAGGTGGCGAGCTGGGACGTGG	6338
-	GGCGCCGT <u>A</u> GTCGCTGC	6339
	GCAGCGAC <u>T</u> ACGGCGCC	6340
Waxy starch GBSS Oryza glaberrima	CGTCCCAGCTCGCCACCTCGGCCACCGGCTTCGGCATCGCTGAC AGGTCGGCGCGTCGTAGCTGCTCCGCCACGGGTTCCAGGGCCT CAAGCCCCGCAGCCCCGCCGGCGGCGACGCGAC	6341
Ser26Term TCG-TAG	GTCGCGTCGCCGCCGGCGGGGCTGCGGGGCTTGAGGCCCTGGA ACCCGTGGCGAGCAGCTACGACGCGCCCGACCTGTCAGCGATG CCGAAGCCGGTGGCCGAGGTGGCGACG	6342
	GCCGTCGTAGCTGCTCC	6343
	GGAGCAGC <u>T</u> ACGACGGC	6344
Waxy starch GBSS Oryza sativa	TCCACAGCAAGAGCTAAACAGCCGACCGTGTGCACCACCATGTCG GCTCTCACCACGTCCTAGCTCGCCACCTCGGCCACCGGCTTCGG CATCGCCGACAGGTCGGCGCCGTCGTCGCTGC	6345
GIn8Term CAG-TAG	GCAGCGACGACGCGCCGACCTGTCGGCGATGCCGAAGCCGGT GGCCGAGGTGGCGAGCTAGGACGTGGTGAGAGCCGACATGGTG GTGCACACGGTCGGCTGTTTAGCTCTTGCTGTGGA	6346

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
	CCACGTCCTAGCTCGCC	6347
	GGCGAGCT <u>A</u> GGACGTGG	6348
Waxy starch GBSS Oryza sativa	CTAAACAGCCGACCGTGTGCACCACCATGTCGGCTCTCACCACGT CCCAGCTCGCCACCTAGGCCACCGGCTTCGGCATCGCCGACAGG TCGGCGCCGTCGTCGCTGCTTCGCCACGGGTT	6349
Ser12Term TCG-TAG	AACCCGTGGCGAAGCAGCGACGACGCGCGACCTGTCGGCGAT GCCGAAGCCGGTGGCCTAGGTGGCGAGCTGGGACGTGGTGAGA GCCGACATGGTGGTGCACACGGTCGGCTGTTTAG	6350
	CGCCACCT <u>A</u> GGCCACCG	6351
	CGGTGGCCTAGGTGGCG	6352
Waxy starch . GBSS Oryza sativa	CGGCTCTCACCACGTCCCAGCTCGCCACCTCGGCCACCGGCTTC GGCATCGCCGACAGGTAGGCGCGTCGTCGCTGCTTCGCCACGG GTTCCAGGGCCTCAAGCCCCGTAGCCCAGCCGG	6353
Ser22Term TCG-TAG	CCGGCTGGGCTACGGGGCTTGAGGCCCTGGAACCCGTGGCGAA GCAGCGACGACGCCCTACCTGTCGGCGATGCCGAAGCCGGT GGCCGAGGTGGCGAGCTGGGACGTGGTGAGAGCCG	6354
	CGACAGGT <u>A</u> GGCGCCGT	6355
	ACGGCGCCTACCTGTCG	6356
Waxy starch GBSS Oryza sativa	CCACGTCCCAGCTCGCCACCTCGGCTTCGGCATCGCC GACAGGTCGGCGCGTAGTCGCTTCGCCACGGGTTCCAGGG CCTCAAGCCCCGTAGCCCAGCCGGCGGGGACGC	6357
Ser25Term TCG-TAG	GCGTCCCGCCGGCTGGGCTACGGGGCTTGAGGCCCTGGAACC CGTGGCGAAGCAGCGACTACGGCGCCCGACCTGTCGGCGATGCC GAAGCCGGTGGCCGAGGTGGCGAGCTGGGACGTGG	6358
	GGCGCCGT <u>A</u> GTCGCTGC	6359
	GCAGCGAC <u>T</u> ACGGCGCC	6360
Waxy starch GBSS Oryza sativa	CGTCCCAGCTCGCCACCTCGGCCACCGGCTTCGGCATCGCCGAC AGGTCGCGCGCGTCGTAGCTGCCACGGGTTCCAGGGCCT CAAGCCCCGTAGCCCAGCCGGCGGGGACGCATC	6361
Ser26Term TCG-TAG	GATGCGTCCCGCCGGCTGGGCTACGGGGCTTGAGGCCCTGGAA CCCGTGGCGAAGCAGCTACGACGCGCGACCTGTCGGCGATGC CGAAGCCGGTGGCCGAGGTGGCGAGCTGGGACG	6362
	GCCGTCGTAGCTGCTTC	6363
	GAAGCAGCTACGACGGC	6364

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ II No:
Waxy starch GBSS Hordeum vulgare	GTCTCTCACTGCAGGTAGCCACACCCTGTGCGCGCGCCATGGC GGCTCTGGCCACGTCCTAGCTCGCCACCTCCGGCACCGTCCTCG GCGTCACCGACAGATTCCGGCGTCCAGGTTTTC	6365
GIn8Term CAG-TAG	GAAAACCTGGACGCCGGAATCTGTCGGTGACGCCGAGGACGGTG CCGGAGGTGGCGAGCTAGGACGTGGCCAGAGCCGCCATGGCGC CGCGCACAGGGTGTGGCTACCTGCAGTGAGAGAC	6366
	CCACGTCCTAGCTCGCC	6367
	GGCGAGCT <u>A</u> GGACGTGG	6368
Waxy starch GBSS Hordeum vulgare	ATGGCGGCTCTGGCCACGTCCCAGCTCGCCACCTCCGGCACCGT CCTCGGCGTCACCGACTGATTCCGGCGTCCAGGTTTTCAGGGCCT CAGGCCCCGGAACCCGGCGGATGCGGCGCTTG	6369
Arg21Term AGA-TGA	CAAGCGCCGCATCCGCCGGGTTCCGGGGCCTGAGGCCCTGAAAA CCTGGACGCCGGAATCAGTCGGTGACGCCGAGGACGTGCCGG AGGTGGCGAGCTGGGACGTGCCAGAGCCGCCAT	6370
	TCACCGAC <u>T</u> GATTCCGG	6371
	CCGGAATC <u>A</u> GTCGGTGA	6372
Waxy starch GBSS Hordeum vulgare	CAGCTCGCCACCTCCGGCACCGTCCTCGGCGTCACCGACAGATT CCGGCGTCCAGGTTTTTAGGGCCTCAGGCCCCGGAACCCGGCGG ATGCGGCGCTTGGTATGAGGACTATCGGAGCAA	6373
GIn28Term CAG-TAG	TTGCTCCGATAGTCCTCATACCAAGCGCCGCATCCGCCGGGTTCC GGGGCCTGAGGCCCTAAAAACCTGGACGCCGGAATCTGTCGGTG ACGCCGAGGACGTGCCGGAGGTGGCGAGCTG	6374
	CAGGTTTT <u>T</u> AGGGCCTC	6375
	GAGGCCCT <u>A</u> AAAACCTG	6376
Waxy starch GBSS Hordeum vulgare	GGTTTTCAGGGCCTCAGGCCCCGGAACCCGGCGATGCGGCGCT TGGTATGAGGACTATCTGAGCAAGCGCCGCCCCGAAGCAAAGCC GGAAAGCGCACCGCGGGAGCCGGCGGTGCCTCT	6377
Gly46Term GGA-TGA	AGAGGCACCGCCGGCTCCCGCGGTGCGCTTTCCGGCTTTGCTTC GGGGCGGCGCTTGCTCAGATAGTCCTCATACCAAGCGCCGCATC CGCCGGGTTCCGGGGCCCTGAGGCCCTGAAAACC	6378
	GGACTATC <u>T</u> GAGCAAGC	6379
	GCTTGCTC <u>A</u> GATAGTCC	6380
Waxy starch GBSS Hordeum vulgare	CCCCGGAACCCGCCGATGCGGCGCTTGGTATGAGGACTATCGG AGCAAGCGCCCCCCGTAGCAAAGCCGGAAAGCGCACCGCGGG AGCCGGCGGTGCCTCTCCGTGGTGGTGAGCGCCA	6381
Lys52Term AAG-TAG	TGGCGCTCACCACGGAGAGGCACCGCCGGCTCCCGCGGTG CGCTTTCCGGCTTTGCTACGGGGCGCGCTTGCTCCGATAGTCC TCATACCAAGCGCCGCATCCGCCGGGTTCCGGGG	6382

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	CCGCCCGTAGCAAAGC	6383
	GCTTTGCT <u>A</u> CGGGGCGG	6384
Waxy starch GBSS Zea mays	ACGTCTTTTCTCTCTCCTACGCAGTGGATTAATCGGCATGGCGG CTCTGGCCACGTCGTAGCTCGTCGCAACGCGCGCCGGCCTGGGC GTCCCGGACGCGTCCACGTTCCGCCGCGGCG	6385
Gin8Term CAG-TAG	CGCCGCGCGAACGTGGACGCCTCCGGGACGCCCAGGCCGGCGCGCGTTGCGACGAGCCAGGCCATGCCGATTAATCCACTGCGTAGGAGAGAGA	6386
	CCACGTCG <u>T</u> AGCTCGTC	6387
	GACGAGCT <u>A</u> CGACGTGG	6388
Waxy starch GBSS Zea mays	GTCGCAACGCGCCCGGCCTGGGCGTCCCGGACGCGTCCACGT TCCGCCGCGCGCGCGCGTAGGGCCTGAGGGGGGCCCGGGCGTC GGCGGCGGCGACACGCTCAGCATGCGGACCAGCG	6389
GIn30Term CAG-TAG	CGCTGGTCCGCATGCTGAGCGTGTCCGCCGCCGCCGACGCCCGGGCGCCCCCCCGACGCCCCGGGCGCGCGCGCGCGCGCGCGCGCGCGCGCGCGC	6390
	GCGCCGCGTAGGGCCTG	6391
	CAGGCCCT <u>A</u> CGCGGCGC	6392
Waxy starch GBSS Zea mays	TCCCGGACGCGTCCACGTTCCGCCGCGCGCGCGCGCGCGC	6393 ·
Ser38Term TCG-TAG	TGCTGGTGCCTGGGCGCGCGCGCGCGCTGGTCCGCATGCTGA GCGTGTCCGCCGCCCCTACGCCCGGGCCCCCTCAGGCCCTG CGCGCGCCGCGC	6394
	CCGGGCGT <u>A</u> GGCGGCGG	6395
	CCGCCGCC <u>T</u> ACGCCCGG	6396
Waxy starch GBSS Zea mays	GCGTCGCCGCGCGCGCACACGCTCAGCATGCGGACCAGCGCGC GCGCGCCCCAGGCACTAGCAGCAGCGCGCGCGGGGGCA GGTTCCCGTCGCTCGTCGTGCGCCAGCGCCGCA	6397
Ser57Term CAG-TAG	TGCCGGCGCTGGCGCACACGACGAGCGACGGGAACCTGCCCCC GCGGCGCGCCTGCTAGTGCCTGGGCGCGCGCGCGCGCGCG	6398
	CCAGGCAC <u>T</u> AGCAGCAG	6399
·	CTGCTGCTAGTGCCTGG	6400

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Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQID NO:
Waxy starch GBSS Zea mays	TCGGCGCGCGACACGCTCAGCATGCGGACCAGCGCGCGCG	6401
GIn58Term CAG-TAG	TCATGCCGGCGCTGGCGCACACGACGACGACGGGAACCTGCCCCCGCGGGCGCGCCCCCGCGCGCG	6402
	GGCACCAG <u>T</u> AGCAGGCG	6403
	CGCCTGCTACTGGTGCC	6404

## Example 11

## Altering fatty acid content of plants

Improved means to manipulate fatty acid compositions, from biosynthetic or natural plant sources, are needed. For example, oils containing reduced saturated fatty acids are desired for dietary reasons and oils containing increased saturated fatty acids are also needed as alternatives to current sources of highly saturated oil products, such as tropical oils or chemically hydrogenated oils. It would therefore be advantageous to influence directly the production and composition of fatty acids in crop plants.

Higher plants synthesize fatty acids, primarily palmitic, stearic and oleic acids, in the plastids (i.e., chloroplasts, proplastids, or other related organelles) as part of the Fatty Acid Synthase (FAS) complex. Fatty acid synthesis is the result of the three enzymatic activities: acyl-ACP elongase, acyl-ACP desaturase and acyl-ACP thioesterases specific for each of palmitoyl-, stearoyl- and oleoyl-ACP.

A variety of enzymes have been identified that influence the relative levels of saturated vs. unsaturated fatty acids in plants. For example, the enzymes stearoyl-acyl carrier protein (stearoyl-ACP) desaturase, oleoyl desaturase and linoleate desaturase produce unsaturated fatty acids from saturated precursors. Similarly, relative enzymatic activities of the various acyl-ACP thioesterases influences the relative acyl-chain composition of the resultant fatty acids. Consequently a reduction or an increase of the activity of these enzymes can alter the properties of oils produced in a plant. In fact, specific targeting of particular enzymatic activities can results in altered levels of particular fatty acids.

The attached tables disclose exemplary oligonucleotides base sequences which can be used to generate site-specific mutations in plant genes encoding proteins involved in fatty acid biosynthesis.

Table 22
Oligonucleotides to produce plants with reduced palmitate

Phenotype, Gene, Plant & Targeted Atteration	Aftering Oligos	SEQ ID NO:
Reduced palmitate Acyl-ACP-thioesterase Arabidopsis thaliana Ser8Term TCG-TAG	TTTGGTGGCAGTGTCTTTGAACGCTTCATCTCCTCGTCATGGTGGC CACCTCTGCTACGTAGTCATTCTTTCCTGTACCATCTTCTTCACTT GATCCTAATGGAAAAGGCAATAAGATTGG	6405
	CCAATCTTATTGCCTTTTCCATTAGGATCAAGTGAAGAAGATGGTA CAGGAAAGAATGACTACGTAGCAGAGGTGGCCACCATGACGAGG AGATGAAGCGTTCAAAGACACTGCCACCAAA	6406

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Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID NO:
	TGCTACGT <u>A</u> GTCATTCT	6407
	AGAATGAC <u>T</u> ACGTAGCA	6408
Reduced palmitate Acyl-ACP-thioesterase Arabidopsis thaliana	GGTGGCAGTGTCTTTGAACGCTTCATCTCCTCGTCATGGTGGCCA CCTCTGCTACGTCGTGATTCTTTCCTGTACCATCTTCTTCACTTGAT CCTAATGGAAAAGGCAATAAGATTGGGTC	6409
Ser9Term TCA-TGA	GACCCAATCTTATTGCCTTTTCCATTAGGATCAAGTGAAGAAGATG GTACAGGAAAGAATCACGACGTAGCAGAGGTGGCCACCATGACGA GGAGATGAAGCGTTCAAAGACACTGCCACC	6410
	TACGTCGTGATTCTTTC	6411
	GAAAGAAT <u>C</u> ACGACGTA	6412
Reduced palmitate Acyl-ACP-thioesterase Arabidopsis thaliana	ATCTCCTCGTCATGGTGGCCACCTCTGCTACGTCGTCATTCTTTCC TGTACCATCTTCTTGACTTGA	6413
Ser17Term TCA-TGA	GAATTGAGTCCAGCAAGATTCGTAGACCCAATCTTATTGCCTTTTC CATTAGGATCAAGTCAAG	6414
	ATCTTCTTGACTTGATC	6415
	GATCAAGT <u>C</u> AAGAAGAT	6416
Reduced palmitate Acyl-ACP-thioesterase Arabidopsis thaliana	GTGGCCACCTCTGCTACGTCGTCATTCTTTCCTGTACCATCTTCTT CACTTGATCCTAATTGAAAAGGCAATAAGATTGGGTCTACGAATCT TGCTGGACTCAATTCTGCACCTAACTCTG	6417
Gly22Term GGA-TGA	CAGAGTTAGGTGCAGAATTGAGTCCAGCAAGATTCGTAGACCCAA TCTTATTGCCTTTTCAATTAGGATCAAGTGAAGAAGATGGTACAGG AAAGAATGACGACGTAGCAGAGGTGGCCAC	6418
	ATCCTAAT <u>T</u> GAAAAGGC	6419
	GCCTTTTC <u>A</u> ATTAGGAT	6420
Reduced palmitate Acyl-ACP-thioesterase Garcinia mangostana	GCTTGAATTTGTGATCTGATTGGTTAATTGTGGCCACAATGGTTGC TACTGCCGCCACGTGATCATTCTTTCCGTTGACTTCCCCTTCTGGG GATGCCAAATCGGGCAATCCCGGAAAAGG	6421
Ser8Term TCA-TGA	CCTTTTCCGGGATTGCCCGATTTGGCATCCCCAGAAGGGGAAGTC AACGGAAAGAATGATCACGTGGCGCAGTAGCAACCATTGTGGCC ACAATTAACCAATCAGATCACAAATTCAAGC	6422
	CGCCACGT <u>G</u> ATCATTCT	6423
	AGAATGAT <u>C</u> ACGTGGCG	6424

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Reduced palmitate Acyl-ACP-thioesterase Garcinia mangostana	GCCAAATCGGGCAATCCCGGAAAAGGGTC	į
Ser9Term TCA-TGA	GACCCTTTTCCGGGATTGCCCGATTTGGCATCCCCAGAAGGGGAA GTCAACGGAAAGAATCATGACGTGGCGGCAGTAGCAACCATTGTG GCCACAATTAACCAATCAGATCACAAATTCA	
	CACGTCATGATCATCA	6427
Dadward - 1-2-1-1	GAAAGAAT <u>C</u> ATGACGTG	6428
Reduced palmitate Acyl-ACP-thioesterase Garcinia mangostana	CTGATTGGTTAATTGTGGCCACAATGGTTGCTACTGCCGCCACGT CATCATTCTTTCCGTAGACTTCCCCTTCTGGGGATGCCAAATCGG GCAATCCCGGAAAAGGGTCGGTGAGTTTTGG	6429
Leu13Term TTG-TAG	CCAAAACTCACCGACCCTTTTCCGGGATTGCCCGATTTGGCATCC CCAGAAGGGGAAGTCTACGGAAAGAATGATGACGTGGCGGCAGT AGCAACCATTGTGGCCACAATTAACCAATCAG	6430
	CTTTCCGTAGACTTCCC	6431
	GGGAAGTC <u>T</u> ACGGAAAG	6432
Reduced palmitate Acyl-ACP-thioesterase Garcinia mangostana	ATGGTTGCTACTGCCGCCACGTCATCATTCTTTCCGTTGACTTCCC CTTCTGGGGATGCCTAATCGGGCAATCCCGGAAAAGGGTCGGTG AGTTTTGGGTCAATGAAGTCGAAATCCGCGG	6433
Lys21Term AAA-TAA	CCGCGGATTTCGACTTCATTGACCCAAAACTCACCGACCCTTTTCC GGGATTGCCCGATTAGGCATCCCCAGAAGGGGAAGTCAACGGAA AGAATGATGACGTGGCGGCAGTAGCAACCAT	6434
	GGGATGCC <u>T</u> AATCGGGC	6435
	GCCCGATT <u>A</u> GGCATCCC	6436
Reduced palmitate Acyl-ACP-thioesterase Gossypium hirsutum	GGGATTICAGCACGAAATTGAAGTTGTTTTTAAAAACCATGGTTGC TACTGCTGTGACATAGGCGTTTTTCCCAGTCACTTCTTCACCTGAC TCCTCTGACTCGAAAAACAAGAAGCTCGG	6437
Ser8Term TCG-TAG	CCGAGCTTCTTGTTTTTCGAGTCAGAGGAGTCAGGTGAAGAAGTG ACTGGGAAAAACGCCTATGTCACAGCAGTAGCAACCATGGTTTTTA AAAACAACTTCAATTTCGTGCTGAAATCCC	6438
	TGTGACAT <u>A</u> GGCGTTTT	6439
	AAAACGCC <u>T</u> ATGTCACA	6440
	TGTTTTAAAAACCATGGTTGCTACTGCTGTGACATCGGCGTTTTT CCCAGTCACTTCTTGACCTGACTCGAAAAACAAGAAG CTCGGAAGCATCAAGTCGAAGCCATCGGT	6441
Ser16Term TCA-TGA	ACCGATGGCTTCGACTTGATGCTTCCGAGCTTCTTGTTTTTCGAGT CAGAGGAGTCAGGTCAAGAAGTGACTGGGAAAAACGCCGATGTCA CAGCAGTAGCAACCATGGTTTTTAAAAACA	6442

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	CACTTCTT <u>G</u> ACCTGACT	6443
	AGTCAGGT <u>C</u> AAGAAGTG	6444
Reduced palmitate Acyl-ACP-thioesterase Gossypium hirsutum	TTGCTACTGCTGTGACATCGGCGTTTTTCCCAGTCACTTCTTCACC TGACTCCTCTGACTAGAAAAACAAGAAGCTCGGAAGCATCAAGTC GAAGCCATCGGTTTCTTCTGGAAGTTTGCA	6445
Ser22Term TCG-TAG	TGCAAACTTCCAGAAGAAACCGATGGCTTCGACTTGATGCTTCCGA GCTTCTTGTTTTTC <u>T</u> AGTCAGAGGAGTCAGGTGAAGAAGTGACTGG GAAAAACGCCGATGTCACAGCAGTAGCAA	6446
	CTCTGACT <u>A</u> GAAAAACA	6447
	TGTTTTC <u>T</u> AGTCAGAG	6448
Reduced palmitate Acyl-ACP-thioesterase Gossypium hirsutum	GCTACTGCTGTGACATCGGCGTTTTTCCCAGTCACTTCTTCACCTG ACTCCTCTGACTCGTAAAACAAGAAGCTCGGAAGCATCAAGTCGA AGCCATCGGTTTCTTCTGGAAGTTTGCAAG	6449
Lys23Term AAA-TAA	CTTGCAAACTTCCAGAAGAAACCGATGGCTTCGACTTGATGCTTCC GAGCTTCTTGTTTTACGAGTCAGAGGAGTCAGGTGAAGAAGTGAC TGGGAAAAACGCCGATGTCACAGCAGTAGC	6450
	CTGACTCG <u>T</u> AAAACAAG	6451
	CTTGTTTT <u>A</u> CGAGTCAG	6452
Reduced palmitate Acyl-ACP-thioesterase Cuphea hookeriana	CTCCCGCTCGTTGAAAGACAATGGTGGCTACCGCTGCAAGCTCTG CATTCTTCCCCGTGTAGTCCCCGGTCACCTCCTCTAGACCAGGAA AGCCCGGAAATGGGTCATCGAGCTTCAGCCC	6453
Ser14Term TCG-TAG	GGGCTGAAGCTCGATGACCCATTTCCGGGCTTTCCTGGTCTAGAG GAGGTGACCGGGGACTACACGGGGAAGAATGCAGAGCTTGCAGC GGTAGCCACCATTGTCTTTCAACGAGCGGGAG	6454
	CCCCGTGTAGTCCCCGG	6455
	CCGGGGACTACACGGGG	6456
Reduced palmitate Acyl-ACP-thioesterase Cuphea hookeriana Arg21Term AGA-TGA	ATGGTGGCTACCGCTGCAAGCTCTGCATTCTTCCCCGTGTCGTCC CCGGTCACCTCCTCTGACCAGGAAAGCCCGGAAATGGGTCATCG AGCTTCAGCCCCATCAAGCCCAAATTTGTCG	6457
	CGACAAATTTGGGCTTGATGGGGCTGAAGCTCGATGACCCATTTC CGGGCTTTCCTGGTCAAGAGGAGGTGACCGGGGACGACACGGG GAAGAATGCAGAGCTTGCAGCGGTAGCCACCAT	6458
	CCTCCTCTTGACCAGGA	6459
	TCCTGGTC <u>A</u> AGAGGAGG	6460

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Reduced palmitate Acyl-ACP-thioesterase Cuphea hookeriana	GCTACCGCTGCAAGCTCTGCATTCTTCCCCGTGTCGTCCCCGGTC ACCTCCTCTAGACCATGAAAGCCCGGAAATGGGTCATCGAGCTTC AGCCCCATCAAGCCCAAATTTGTCGCCAATG	6461
Gly23Term GGA-TGA	CATTGGCGACAAATTTGGGCTTGATGGGGCTGAAGCTCGATGACC CATTTCCGGGCTTTCATGGTCTAGAGGAGGTGACCGGGGACGAC ACGGGGAAGAATGCAGAGCTTGCAGCGGTAGC	6462
	CTAGACCA <u>T</u> GAAAGCCC	6463
	GGGCTTTC <u>A</u> TGGTCTAG	6464
Reduced palmitate Acyl-ACP-thioesterase Cuphea hookeriana	ACCGCTGCAAGCTCTGCATTCTTCCCCGTGTCGTCCCCGGTCACC TCCTCTAGACCAGGATAGCCCGGAAATGGGTCATCGAGCTTCAGC CCCATCAAGCCCAAATTTGTCGCCAATGGCG	6465
Lys24Term AAG-TAG	CGCCATTGGCGACAAATTTGGGCTTGATGGGGCTGAAGCTCGATG ACCCATTTCCGGGCTATCCTGGTCTAGAGGAGGTGACCGGGGAC GACACGGGGAAGAATGCAGAGCTTGCAGCGGT	6466
	GACCAGGA <u>T</u> AGCCCGGA	6467
	TCCGGGCTATCCTGGTC	6468
Reduced palmitate Acyl-ACP-thioesterase Cuphea lanceolata	GCCACCGCTGCAAGTTCTGCATTCTTCCCCCTGCCGTCCCCGGAC ACCTCCTCTAGGCCGTGAAAGCTCGGAAATGGGTCATCGAGCTTG AGCCCCCTCAAGCCCAAATTTGTCGCCAATG	6469
Gly23Term GGA-TGA	CATTGGCGACAAATTTGGGCTTGAGGGGGGCTCAAGCTCGATGACC CATTTCCGAGCTTTCACGGCCTAGAGGAGGTGTCCGGGGACGGC AGGGGGAAGAATGCAGAACTTGCAGCGGTGGC	6470
	CTAGGCCG <u>T</u> GAAAGCTC	6471
	GAGCTTTCACGCCTAG	6472
Reduced palmitate Acyl-ACP-thioesterase Cuphea lanceolata	ACCGCTGCAAGTTCTGCATTCTTCCCCCTGCCGTCCCCGGACACC TCCTCTAGGCCGGGATAGCTCGGAAATGGGTCATCGAGCTTGAGC CCCCTCAAGCCCAAATTTGTCGCCAATGCCG	6473
Lys24Term AAG-TAG	CGGCATTGGCGACAAATTTGGGCTTGAGGGGGGCTCAAGCTCGAT GACCCATTTCCGAGCTAATCCCGGCCTAGAGGAGGTGTCCGGGGA CGGCAGGGGGAAGAATGCAGAACTTGCAGCGGT	6474
	GGCCGGGA <u>T</u> AGCTCGGA	6475
	TCCGAGCT <u>A</u> TCCCGGCC	6476
Reduced palmitate Acyl-ACP-thioesterase Cuphea lanceolata	GCAAGTTCTGCATTCTTCCCCCTGCCGTCCCCGGACACCTCCTCT AGGCCGGGAAAGCTCTGAAATGGGTCATCGAGCTTGAGCCCCCT CAAGCCCAAATTTGTCGCCAATGCCGGGTTGA	6477
Gly26Term GGA-TGA	TCAACCCGGCATTGGCGACAAATTTGGGCTTGAGGGGGCTCAAGC TCGATGACCCATTTCAGAGGCTTTCCCGGCCTAGAGGAGGTGTCCG GGGACGGCAGGGGAAGAATGCAGAACTTGC	6478

Phenotype, Gene. Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	GAAAGCTC <u>T</u> GAAATGGG	6479
	CCCATTTC <u>A</u> GAGCTTTC	6480
Reduced palmitate Acyl-ACP-thioesterase Cuphea lanceolata	CATTCTTCCCCCTGCCGTCCCCGGACACCTCCTCTAGGCCGGGAA AGCTCGGAAATGGGTGATCGAGCTTGAGCCCCCTCAAGCCCAAAT TTGTCGCCAATGCCGGGTTGAAGGTTAAGGC	6481
Ser29Term TCA-TGA	GCCTTAACCTTCAACCCGGCATTGGCGACAAATTTGGGCTTGAGG GGGCTCAAGCTCGAT <u>C</u> ACCCATTTCCGAGCTTTCCCGGCCTAGAG GAGGTGTCCGGGGACGGCAGGGGGAAGAATG	6482
	AAATGGGT <u>G</u> ATCGAGCT	6483
	AGCTCGAT <u>C</u> ACCCATTT	6484
Reduced palmitate Acyl-ACP-thioesterase Helianthus annuus	CGTTTAAGTGGATCGGACATTTAAGTGTTTTAATCATGGTAGCTAT GAGTGCTACTGCGTAGCTGTTTCCGGTTTCTTCCCCAAAACCTCA CTCTGGAGCCAAGACATCTGATAAGCTTGG	6485
Ser9Term TCG-TAG	CCAAGCTTATCAGATGTCTTGGCTCCAGAGTGAGGTTTTGGGGAA GAAACCGGAAACAGC <u>T</u> ACGCAGTAGCACTCATAGCTACCATGATT AAAACACTTAAATGTCCGATCCACTTAAACG	6486
	TACTGCGTAGCTGTTTC	6487
	GAAACAGC <u>T</u> ACGCAGTA	6488
Reduced palmitate Acyl-ACP-thioesterase Helianthus annuus	AGTGTTTTAATCATGGTAGCTATGAGTGCTACTGCGTCGCTGTTTC CGGTTTCTTCCCCATAACCTCACTCTGGAGCCAAGACATCTGATAA GCTTGGAGGTGAACCAGGTAGTGTTGCTG	6489
Lys17Term AAA-TAA	CAGCAACACTACCTGGTTCACCTCCAAGCTTATCAGATGTCTTGGC TCCAGAGTGAGGTTATGGGGAAGAAACCGGAAACAGCGACGCAG TAGCACTCATAGCTACCATGATTAAAACACT	6490
	CTTCCCCA <u>T</u> AACCTCAC	6491
	GTGAGGTT <u>A</u> TGGGGAAG	6492
Reduced palmitate Acyl-ACP-thioesterase Helianthus annuus	ATGGTAGCTATGAGTGCTACTGCGTCGCTGTTTCCGGTTTCTTCCC CAAAACCTCACTCTTGAGCCAAGACATCTGATAAGCTTGGAGGTGA ACCAGGTAGTGTTGCTGTGCGCGGAATCA	6493
Gly21Term GGA-TGA	TGATTCCGCGCACAGCAACACTACCTGGTTCACCTCCAAGCTTATC AGATGTCTTGGCTCAAGAGTGAGGTTTTGGGGAAGAAACCGGAAA CAGCGACGCAGTAGCACTCATAGCTACCAT	6494
	CTCACTCT <u>T</u> GAGCCAAG	6495
	CTTGGCTC <u>A</u> AGAGTGAG	6496

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Reduced palmitate Acyl-ACP-thioesterase Helianthus annuus	GCTATGAGTGCTACTGCGTCGCTGTTTCCGGTTTCTTCCCCAAAAC CTCACTCTGGAGCCTAGACATCTGATAAGCTTGGAGGTGAACCAG GTAGTGTTGCTGTGCGCGGAATCAAGACAA	6497
Lys23Term AAG-TAG	TTGTCTTGATTCCGCGCACAGCAACACTACCTGGTTCACCTCCAAG CTTATCAGATGTCTAGGCTCCAGAGTGAGGTTTTGGGGAAGAAAC CGGAAACAGCGACGCAGTAGCACTCATAGC	6498
	CTGGAGCC <u>T</u> AGACATCT AGATGTCT <u>A</u> GGCTCCAG	6499
Reduced palmitate	<u> </u>	6500
Acyl-ACP-thioesterase Cuphea palustris	ATGGTGGCTGCAGCAAGTTCTGCATGCTTCCCTGTTCCATCC CCAGGAGCCTCCCCTTAACCTGGGAAGTTAGGCAACTGGTCATCG AGTTTGAGCCCTTCCTTGAAGCCCAAGTCAA	6501
Lys21Term AAA-TAA	TTGACTTGGGCTTCAAGGAAGGGCTCAAACTCGATGACCAGTTGC CTAACTTCCCAGGTTAAGGGGAGGCTCCTGGGGATGGAACAGGG AAGCATGCAGAACTTGCTGCAGCAGCCACCAT	6502
	CCTCCCCT <u>T</u> AACCTGGG	6503
	CCCAGGTT <u>A</u> AGGGGAGG	6504
Reduced palmitate Acyl-ACP-thioesterase Cuphea palustris	GCTGCAGCAAGTTCTGCATGCTTCCCTGTTCCATCCCCAGGAGCC TCCCCTAAACCTGGGTAGTTAGGCAACTGGTCATCGAGTTTGAGC CCTTCCTTGAAGCCCAAGTCAATCCCCAATG	6505
Lys24Term AAG-TAG	CATTGGGGATTGACTTGGGCTTCAAGGAAGGGCTCAAACTCGATG ACCAGTTGCCTAACTACCCAGGTTTAGGGGAGGCTCCTGGGGATG GAACAGGGAAGCATGCAGAACTTGCTGCAGC	6506
	AACCTGGG <u>T</u> AGTTAGGC	6507
	GCCTAACT <u>A</u> CCCAGGTT	6508
Reduced palmitate Acyl-ACP-thioesterase Cuphea palustris	TGCATGCTTCCCTGTTCCATCCCCAGGAGCCTCCCCTAAACCTGG GAAGTTAGGCAACTGATCATCGAGTTTGAGCCCTTCCTTGAAGCC CAAGTCAATCCCCAATGGCGGATTTCAGGTT	6509
Trp28Term TGG-TGA	AACCTGAAATCCGCCATTGGGGATTGACTTGGGCTTCAAGGAAGG	6510
	GGCAACTG <u>A</u> TCATCGAG	6511
	CTCGATGA <u>T</u> CAGTTGCC	6512
Reduced palmitate Acyl-ACP-thioesterase Cuphea palustris	CATGCTTCCCTGTTCCATCCCCAGGAGCCTCCCCTAAACCTGGGA AGTTAGGCAACTGGTGATCGAGTTTGAGCCCTTCCTTGAAGCCCA AGTCAATCCCCAATGGCGGATTTCAGGTTAA	6513
Ser29Term TCA-TGA	TTAACCTGAAATCCGCCATTGGGGATTGACTTGGGCTTCAAGGAA GGGCTCAAACTCGATCACCAGTTGCCTAACTTCCCAGGTTTAGGG GAGGCTCCTGGGGATGGAACAGGGAAGCATG	6514

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	CAACTGGT <u>G</u> ATCGAGTT	6515
	AACTCGAT <u>C</u> ACCAGTTG	6516
Reduced palmitate	ATGGTGGCTGCCGCAGCAAGTTCTGCATTCTTCTCCGTTCCAACC	6517
Acyl-ACP-thioesterase	CCGGGAATCTCCCCTTAACCCGGGAAGTTCGGTAATGGTGGCTTT	
Cuphea hookeriana	CAGGTTAAGGCAAACGCCAATGCCCATCCTA	27.4
Lys21Term	TAGGATGGCATTGCCTTAACCTGAAAGCCACCATTAC	6518
AAA-TAA	CGAACTTCCCGGGTTAAGGGGAGATTCCCGGGGTTGGAACGGAG	
	AAGAATGCAGAACTTGCTGCGGCAGCCACCAT	0540
	TCTCCCCTTAACCCGGG	6519
	CCCGGGTT <u>A</u> AGGGGAGA	6520
Reduced palmitate	GCCGCAGCAAGTTCTGCATTCTTCTCCGTTCCAACCCCGGGAATC	6521
Acyl-ACP-thioesterase	TCCCCTAAACCCGGG <u>T</u> AGTTCGGTAATGGTGGCTTTCAGGTTAAG	
Cuphea hookeriana	GCAAACGCCAATGCCCATCCTAGTCTAAAGT	
Lys24Term	ACTITAGACTAGGATGGCCATTGGCGTTTGCCTTAACCTGAAAGCC	6522
AAG-TAG	ACCATTACCGAACT <u>A</u> CCCGGGTTTAGGGGAGATTCCCGGGGTTGG	
	AACGGAGAAGAATGCAGAACTTGCTGCGGC	
	AACCCGGG <u>T</u> AGTTCGGT	6523
	ACCGAACT <u>A</u> CCCGGGTT	6524
Reduced palmitate	TTCTCCGTTCCAACCCCGGGAATCTCCCCTAAACCCGGGAAGTTC	6525
Acyl-ACP-thioesterase	GGTAATGGTGGCTTT <u>T</u> AGGTTAAGGCAAACGCCAATGCCCATCCT	
Cuphea hookeriana	AGTCTAAAGTCTGGCAGCCTCGAGACTGAAG	
Gin31Term	CTTCAGTCTCGAGGCTGCCAGACTTTAGACTAGGATGGGCATTGG	6526
CAG-TAG	CGTTTGCCTTAACCTAAAAGCCACCATTACCGAACTTCCCGGGTTT	
	AGGGGAGATTCCCGGGGTTGGAACGGAGAA	
:	GTGGCTTT <u>T</u> AGGTTAAG	6527
·	CTTAACCT <u>A</u> AAAGCCAC	6528
Reduced palmitate	GTTCCAACCCGGGAATCTCCCCTAAACCCGGGAAGTTCGGTAAT	6529
Acyl-ACP-thioesterase	GGTGGCTTTCAGGTT <u>T</u> AGGCAAACGCCAATGCCCATCCTAGTCTA	
Cuphea hookeriana	AAGTCTGGCAGCCTCGAGACTGAAGATGACA	
Lys33Term	TGTCATCTTCAGTCTCGAGGCTGCCAGACTTTAGACTAGGATGGG	6530
AAG-TAG	CATTGGCGTTTGCCTAAACCTGAAAGCCACCATTACCGAACTTCCC	
	GGGTTTAGGGGAGATTCCCGGGGTTGGAAC	
	TTCAGGTTTAGGCAAAC	6531
	GTTTGCCT <u>A</u> AACCTGAA	6532

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID No:
Reduced palmitate Acyl-ACP-thioesterase Brassica rapa	ATGITGAAGCTCTCGTGTAATGCGACTGATAAGTTACAGACCCTCT TCTCGCATTCTCATTAACCGGATCCGGCACACCGGAGAACCGTCT CCTCCGTGTCGTGCTCTCATCTGAGGAAAC	6533
Gin21Term CAA-TAA	GTITCCTCAGATGAGAGCACGACACGGAGGAGACGGTTCTCCGGT GTGCCGGATCCGGTTAATGAGAATGCGAGAAGAGGGTCTGTAACT TATCAGTCGCATTACACGAGAGCTTCAACAT	6534
	ATTCTCAT <u>T</u> AACCGGAT ATCCGGTT <u>A</u> ATGAGAAT	6535
Reduced palmitate	GCGACTGATAAGTTACAGACCCTCTTCTCGCATTCTCATCAACCGG	6536 6537
Acyl-ACP-thioesterase  Brassica rapa	ATCCGGCACACCGGTGAACCGTCTCCTCCGTGTCGTGCTCTCATC TGAGGAAACCGGTTCTCGATCCTTTGCGAG	
Arg28Term AGA-TGA	CTCGCAAAGGATCGAGAACCGGTTTCCTCAGATGAGAGCACGACA CGGAGGAGACGGTTCACCGGTGCCGGATCCGGTTGATGAGAA TGCGAGAAGAGGGTCTGTAACTTATCAGTCGC	6538
	CACACCGG <u>T</u> GAACCGTC	6539
***	GACGGTTCACCGGTGTG	6540
Reduced palmitate Acyl-ACP-thioesterase Brassica rapa	CCCTCTTCTCGCATTCTCATCAACCGGATCCGGCACACCGGAGAA CCGTCTCCCGTGTAGTGCTCTCATCTGAGGAAACCGGTTCTCG ATCCTTTGCGAGCGATCGTATCTGCTGATCA	6541
Ser24Term TCG-TAG	TGATCAGCAGATACGATCGCTCGCAAAGGATCGAGAACCGGTTTC CTCAGATGAGAGCACTACACGGAGGAGACGGTTCTCCGGTGTGC CGGATCCGGTTGATGAGAATGCGAGAAGAGGG	6542
	CTCCGTGT <u>A</u> GTGCTCTC	6543
	GAGAGCAC <u>T</u> ACACGGAG	6544
Reduced palmitate Acyl-ACP-thioesterase Brassica rapa	CTTCTCGCATTCTCATCAACCGGATCCGGCACACCGGAGAACCGT CTCCTCCGTGTCGTGATCTCATCTGAGGAAACCGGTTCTCGATCC TTTGCGAGCGATCGTATCTGCTGATCAAGGA	6545
Cys25Term TGC-TGA	TCCTTGATCAGCAGATACGATCGCTCGCAAAGGATCGAGAACCGG TTTCCTCAGATGAGATCACGACACGGAGGAGACGGTTCTCCGGTG TGCCGGATCCGGTTGATGAGAATGCGAGAAG	6546
	GTGTCGTG <u>A</u> TCTCATCT	6547
	AGATGAGA <u>T</u> CACGACAC	6548
Acyl-ACP-thioesterase Brassica napus	ATTCTTCTATAAACCAAAACCTCAGGAACCATAAAAAAAA	6549
Leu2Term ITG-TAG	GAGGAATCGGAGAAGAAGGAGAAGGTGTGTAAGTTGTTAGTCACA TTACACGAAAGCTTC <u>T</u> ACATTTTTGATGCCCTTTTTTTTTATGGTTC CTGAGGTTTTGGTTTATAGAAGAAGAAT	6550

Phenotype, Gene, Plant & Targeted Alteration	Attering Oligos	SEQID NO:
	AAAAATGT <u>A</u> GAAGCTTT	6551
	AAAGCTTC <u>T</u> ACATTTTT	6552
Reduced palmitate Acyl-ACP-thioesterase Brassica napus	TCTTCTTCTATAAACCAAAACCTCAGGAACCATAAAAAAAA	6553
Lys3Term AAG-TAG	GGGAGGAATCGGAGAAGAAGGAGAAGGTGTTAAGTTAGTCA CATTACACGAAAGCTACAACATTTTTGATGCCCTTTTTTTT	6554
	AAATGTTG <u>T</u> AGCTTTCG	6555
	CGAAAGCT <u>A</u> CAACATTT	6556
Reduced palmitate Acyl-ACP-thioesterase Brassica napus	CTATAAACCAAAACCTCAGGAACCATAAAAAAAAAAAGGGCATCAAA AATGTTGAAGCTTT <u>A</u> GTGTAATGTGACTAACAACTTACACACCTTCT CCTTCTTCTCCGATTCCTCCCTTTTCAT	6557
Ser5Term TCG-TAG	ATGAAAAGGGAGGAATCGGAGAAGAAGGAGAAGGTGTGAAGTTG TTAGTCACATTACACTAAAGCTTCAACATTTTTGATGCCCTTTTTTTT	6558
	GAAGCTTT <u>A</u> GTGTAATG	6559
	CATTACAC <u>T</u> AAAGCTTC	6560
Reduced palmitate Acyl-ACP-thioesterase Brassica napus	AAACCAAAACCTCAGGAACCATAAAAAAAAAAAGGGCATCAAAAATG TTGAAGCTTTCGTGAAATGTGACTAACAACTTACACACCTTCTCCTT CTTCTCCGATTCCTCCCTTTTCATCCCG	6561
Cys6Term TGT-TGA	CGGGATGAAAAGGGAGGAATCGGAGAAGGAAGGAGAAGGTGTGTA AGTTGTTAGTCACATTTCACGAAAGCTTCAACATTTTTGATGCCCTT TTTTTTTTATGGTTCCTGAGGTTTTGGTTT	6562
	CTTTCGTG <u>A</u> AATGTGAC	6563
į	GTCACATT <u>T</u> CACGAAAG	6564

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Table 23
Oligonucleotides to produce plants with increased stearate

5	Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	Increased stearate stearoyl-ACP desaturase Arabidopsis thaliana	GGGAGAGCTCTAGCTCTGTAGAAAAGAAGGATTCATTCAT	6565
10	Lys4Term AAG-TAG	TTGGCGGACGAGTCGAGGAAGGGAATTTGTAAGGCTGAGATGCCA CCAAAGGGTTAAACTATAGAGCCATTTCTGGATATGATGAAT CCTTCTTTTCTACAGAGCTAGAGCTCTCCC	
		TGGCTCTA <u>T</u> AGTTTAAC GTTAAACT <u>A</u> TAGAGCCA	6567
			6568
45	Increased stearate stearoyl-ACP desaturase	CTCTGTAGAAAAGAAGGATTCATTCATCATATCCAGAAATGGCTCT AAAGTTTAACCCTTAGGTGGCATCTCAGCCTTACAAATTCCCTTCC TCGACTCGTCCGCCAACTCCTTCTTTCAG	6569
<b>15</b>	Arabidopsis thaliana Leu8Term TTG-TAG	CTGAAAGAAGGAGTTGGCGGACGAGTCGAGGAAGGGAATTTGTAA GGCTGAGATGCCACCTAAGGGTTAAACTTTAGAGCCATTTCTGGAT ATGATGAATGAATCCTTCTTTTCTACAGAG	6570
••	,	TAACCCTT <u>A</u> GGTGGCAT	6571
	<u> </u>	ATGCCACC <u>T</u> AAGGGTTA	6572
20	Increased stearate stearoyl-ACP desaturase	AGAAGGATTCATCATCATATCCAGAAATGGCTCTAAAGTTTAACC CTTTGGTGGCATCTTAGCCTTACAAATTCCCTTCCTCGACTCGTCC GCCAACTCCTTCTTTCAGATCTCCCAAGT	6573
	Arabidopsis thaliana GIn12Term CAG-TAG	ACTTGGGAGATCTGAAAGAAGGAGTTGGCGGACGAGTCGAGGAA GGGAATTTGTAAGGCTAAGATGCCACCAAAGGGTTAAACTTTAGAG CCATTTCTGGATATGAATGAATCCTTCT	6574
		TGGCATCT <u>T</u> AGCCTTAC	6575
		GTAAGGCT <u>A</u> AGATGCCA	6576
25	Increased stearate stearoyl-ACP desaturase	TCATTCATCATATCCAGAAATGGCTCTAAAGTTTAACCCTTTGGTG GCATCTCAGCCTTAGAAATTCCCTTCCTCGACTCGTCCGCCAACTC CTTCTTTCAGATCTCCCAAGTTCCTCTGC	6577
	Arabidopsis thaliana Phe14Term TAC-TAG	GCAGAGGAACTTGGGAGATCTGAAAGAAGAGGAGTTGGCGGACGAG TCGAGGAAGGGAATTTCTAAGGCTGAGATGCCACCAAAGGGTTAA ACTTTAGAGCCATTTCTGGATATGATGAATGA	6578
		CAGCCTTAGAAATTCCC	6579
		GGGAATTT <u>C</u> TAAGGCTG	6580

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ III NO:
Increased stearate stearoyl-ACP desaturase	GAGAGCTCGCTCGTGTCTGAAAGAACATCAAACCTCGTATCAAAAA AAAGAAAATGGCATAGAAGCTTAACCCTTTGGCATCTCAGCCTTAC AAACTCCCTTCCTCGGCTCGCCCAAT	6581
Brassica napus Leu3Term TTG-TAG	ATTGGCGGACGAGCCGAGGAAGGGAGTTTGTAAGGCTGAGATGC CAAAGGGTTAAGCTTCTATGCCATTTTCTTTTTTTTGATACGAGGTT TGATGTTCTTTCAGACACGAGCGAGCTCTC	6582
	AATGGCAT <u>A</u> GAAGCTTA	6583
	TAAGCTTC <u>T</u> ATGCCATT	6584
Increased stearate stearoyl-ACP desaturase	GAGCTCGCTCGTGTCTGAAAGAACATCAAACCTCGTATCAAAAAAA AGAAAATGGCATTGTAGCTTAACCCTTTGGCATCTCAGCCTTACAA ACTCCCTTCCTCGGCTCGTCCGCCAATCT	6585
Brassica napus Lys4Term AAG-TAG	AGATTGGCGGACGAGCCGAGGAAGGGAGTTTGTAAGGCTGAGAT GCCAAAGGGTTAAGCTACAATGCCATTTTCTTTTTTTTGATACGAG GTTTGATGTTCTTTCAGACACGAGCGAGCTC	6586
	TGGCATTG <u>T</u> AGCTTAAC	6587
	GTTAAGCT <u>A</u> CAATGCCA	6588
Increased stearate stearoyl-ACP desaturase	TCTGAAAGAACATCAAACCTCGTATCAAAAAAAAAGAAAATGGCATT GAAGCTTAACCCTTAGGCATCTCAGCCTTACAAACTCCCTTCCTCG GCTCGTCCGCCAATCTCTACTCTCAGATC	6589
Brassica napus Leu8Term TTG-TAG	GATCTGAGAGTAGAGATTGGCGGACGAGCCGAGGAAGGGAGTTT GTAAGGCTGAGATGCCTAAGGGTTAAGCTTCAATGCCATTTTCTTT TTTTTGATACGAGGTTTGATGTTCTTTCAGA	6590
	TAACCCTT <u>A</u> GGCATCTC	6591
	GAGATGCC <u>T</u> AAGGGTTA	6592
Increased stearate stearoyl-ACP desaturase	AACATCAAACCTCGTATCAAAAAAAAGAAAATGGCATTGAAGCTTAA CCCTTTGGCATCTTAGCCTTACAAACTCCCTTCCTCGGCTCGTCCG CCAATCTCTACTCTCAGATCTCCCAAGT	6593
Brassica napus Gln11Term CAG-TAG	ACTTGGGAGATCTGAGAGTAGAGATTGGCGGACGAGCCGAGGAA GGGAGTTTGTAAGGCTAAGATGCCAAAGGGTTAAGCTTCAATGCC ATTTTCTTTTTTTGATACGAGGTTTGATGTT	6594
	TGGCATCT <u>T</u> AGCCTTAC	6595
	GTAAGGCT <u>A</u> AGATGCCA	6596
Increased stearate stearoyl-ACP desaturase	AACCAAAAGAAAAAGGTAAGAAAAAAAAAACAATGGCTCTCAAGCTCA ATCCTTTCCTT	6597
<i>Ricinus communis</i> Gln27Term CAA-TAA	ACTTAGGAGATCTGGTACTGGCCATTGGTGGAAGAGCGAAAGAAG GTAACTTTTGGGTTT <u>A</u> AGAAAGGAAAGGATTGAGCTTGAGAGCCAT TGTTTTTTTTCTTACCTTTTTCTTTTGGTT	6598

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	TCCTTTCT <u>T</u> AAACCCAA	6599
	TTGGGTTT <u>A</u> AGAAAGGA	6600
Increased stearate stearoyl-ACP desaturase	AAGAAAAAGGTAAGAAAAAAAAAACAATGGCTCTCAAGCTCAATCCTT TCCTTTCTCAAACCTAAAAGTTACCTTCTTTCGCTCTTCCACCAATG GCCAGTACCAGATCTCCTAAGTTCTACA	6601
Ricinus communis Gln29Term CAA-TAA	TGTAGAACTTAGGAGATCTGGTACTGGCCATTGGTGGAAGAGCGA AAGAAGGTAACTTTTAGGTTTGAGAAAGGAAAG	6602
	CTCAAACC <u>T</u> AAAAGTTA	6603
	TAACTITIAGGTTTGAG	6604
Increased stearate stearoyl-ACP desaturase	AAAAAGGTAAGAAAAAAAAACAATGGCTCTCAAGCTCAATCCTTTCC TTTCTCAAACCCAA <u>T</u> AGTTACCTTCTTTCGCTCTTCCACCAATGGC CAGTACCAGATCTCCTAAGTTCTACATGG	6605
Ricinus communis Lys30Term AAG-TAG	CCATGTAGAACTTAGGAGATCTGGTACTGGCCATTGGTGGAAGAG CGAAAGAAGGTAACTATTGGGTTTGAGAAAGGAAAG	6606
	AAACCCAA <u>T</u> AGTTACCT	6607
	AGGTAACT <u>A</u> TTGGGTTT	6608
Increased stearate stearoyl-ACP desaturase	TCTCAAACCCAAAAGTTACCTTCTTTCGCTCTTCCACCAATGGCCA GTACCAGATCTCCTTAGTTCTACATGGCCTCTACCCTCAAGTCTGG TTCTAAGGAAGTTGAGAATCTCAAGAAGC	6609
Ricinus communis Lys46Term AAG-TAG	GCTTCTTGAGATTCTCAACTTCCTTAGAACCAGACTTGAGGGTAGA GGCCATGTAGAACTAAGGAGATCTGGTACTGGCCATTGGTGGAAG AGCGAAAGAAGGTAACTTTTGGGTTTGAGA	6610
	GATCTCCT <u>T</u> AGTTCTAC	6611
	GTAGAACT <u>A</u> AGGAGATC	6612
stearoyl-ACP desaturase	TCTTCTGATTCATTTAATCTTTACTCATCAATGGCTCTGAGACTGAA CCCTATCCCCACCTAAACCTTCTCCCTCCCCAAATGGCCAGTCT CAGATCTCCCAGGTTCCGCATGGCCTCTA	6613
Glycine max Gln11Term CAA-TAA	TAGAGGCCATGCGGAACCTGGGAGATCTGAGACTGGCCATTTGG GGGAGGAGAAGGTTTAGGTGGGGATAGGGTTCAGTCTCAGAGC CATTGATGAGTAAAGATTAAATGAATCAGAAGA	6614
	TCCCCACC <u>T</u> AAACCTTC	6615
	GAAGGTTT <u>A</u> GGTGGGGA	6616

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Increased stearate stearoyl-ACP desaturase	CTTTACTCATCAATGGCTCTGAGACTGAACCCTATCCCCACCCA	6617
Glycine max Gln17Term CAA-TAA	TGGAACCGGAGCGAGGGTAGAGGCCATGCGGAACCTGGGAGAT CTGAGACTGGCCATTTAGGGGAGGGAGAAGGTTTGGGTGGG	6618
	CCCTCCCCTAAATGGCC	6619
	GGCCATTT <u>A</u> GGGGAGGG	6620
Increased stearate stearoyl-ACP desaturase	GCTCTGAGACTGAACCCTATCCCCACCCAAACCTTCTCCCTCC	6621
Glycine max Arg22Term AGA-TGA	TATTTCAACCTCTTTGGAACCGGAGCGGAGGGTAGAGGCCATGC GGAACCTGGGAGATCAGAGACTGGCCATTTGGGGGAGGGA	6622
	CCAGTCTC <u>T</u> GATCTCCC	6623
	GGGAGATC <u>A</u> GAGACTGG	6624
Increased stearate stearoyl-ACP desaturase	CAAATGGCCAGTCTCAGATCTCCCAGGTTCCGCATGGCCTCTACC CTCCGCTCCG	6625
Glycine max Lys37Term AAA-TAA	TTACTTGAACATGCACTTCTCTGGGAGGAGTGAATGGCTTCTTAAT ATTTTCAACCTCTTAGGAACCGGAGCGAGGGTAGAGGCCATGCG GAACCTGGGAGATGTGAGACTGGCCATTTG	6626
	CCGGTTCC <u>T</u> AAGAGGTT	6627
	AACCTCTT <u>A</u> GGAACCGG	6628
Increased stearate stearoyl-ACP desaturase	CAACAAGCACACAAGAACAACATCAACAATGGCGATTCGCATCA ATACGGCGACGTTTTAATCAGACCTGTACCGTTCATTCGCGTTTCC TCAACCGAAACCTCTCAGATCTCCCAAAT	6629
Helianthus annuus Gin11Term CAA-TAA	ATTTGGGAGATCTGAGAGGTTTCGGTTGAGGAAACGCGAATGAAC GGTACAGGTCTGATTAAAACGTCGCCGTATTGATGCGAATCGCCA TTGTTGATGTTGTTCTTGTGTGTGCTTGTTG	6630
	CGACGTTT <u>T</u> AATCAGAC	6631
	GTCTGATT <u>A</u> AAACGTCG	6632
Increased stearate stearoyl-ACP desaturase	AAGCACACAAGAACAACATCAACAATGGCGATTCGCATCAATAC GGCGACGTTTCAATGAGACCTGTACCGTTCATTCGCGTTTCCTCAA CCGAAACCTCTCAGATCTCCCAAATTCGC	6633
Helianthus annuus Ser12Term TCA-TGA	GCGAATTTGGGAGATCTGAGAGGTTTCGGTTGAGGAAACGCGAAT GAACGGTACAGGTCTCATTGAAACGTCGCCGTATTGATGCGAATC GCCATTGTTGATGTTGTTCTTGTGTGTGCTT	6634

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
	GTTTCAAT <u>G</u> AGACCTGT	6635
	ACAGGTCT <u>C</u> ATTGAAAC	6636
Increased stearate stearoyl-ACP desaturase	AAGAACAACATCAACAATGGCGATTCGCATCAATACGGCGACGTTT CAATCAGACCTGTAGCGTTCATTCGCGTTTCCTCAACCGAAACCTC TCAGATCTCCCAAATTCGCCATGGCTTCC	6637
Helianthus annuus Tyr15Term TAC-TAG	GGAAGCCATGGCGAATTTGGGAGATCTGAGAGGTTTCGGTTGAGG AAACGCGAATGAACGCTACAGGTCTGATTGAAACGTCGCCGTATT GATGCGAATCGCCATTGTTGATGTTGTTCTT	6638
	GACCTGTAGCGTTCATT	6639
	AATGAACG <u>C</u> TACAGGTC	6640
Increased stearate stearoyl-ACP desaturase	CAACATCAACAATGGCGATTCGCATCAATACGGCGACGTTTCAATC AGACCTGTACCGTTGATTCGCGTTTCCTCAACCGAAACCTCTCAGA TCTCCCAAATTCGCCATGGCTTCCACCAT	6641
Helianthus annuus Ser17Term TCA-TGA	ATGGTGGAAGCCATGGCGAATTTGGGAGATCTGAGAGGTTTCGGT TGAGGAAACGCGAATCAACGGTACAGGTCTGATTGAAACGTCGCC GTATTGATGCGAATCGCCATTGTTGATGTTG	6642
•	GTACCGTT <u>G</u> ATTCGCGT	6643
	ACGCGAAT <u>C</u> AACGGTAC	6644
Increased stearate stearoyl-ACP desaturase	ACACACACACACACTCAATCACACACACATCATCTTCTTC	6645
Helianthus annuus Arg4Term CGA-TGA	TCGATTGATGAAAAGTGTATGAAGGATATATCTCCCGTTGAAGCGT CACCGGACTCATTCAAAGCGCCATCGTTGATGAAGAAGATGATGA TGTGTGTGTGATTGAGTGTGTGT	6646
	TGGCGCTT <u>T</u> GAATGAGT	6647
	ACTCATTC <u>A</u> AAGCGCCA	6648
increased stearate stearoyl-ACP desaturase	ACACACACATCATCTTCTTCATCAACGATGGCGCTTCGAATGA GTCCGGTGACGCTTTAACGGGAGATATATCCTTCATACACTTTTCA TCAATCGAAAAATCTCAGATCTCCTAAAT	6649
Helianthus annuus Gln11Term CAA-TAA	ATTTAGGAGATCTGAGATTTTTCGATTGAAAAGTGTATGAAGG ATATATCTCCCGTTAAAGCGTCACCGGACTCATTCGAAGCGCCAT CGTTGATGAAGAAGATGATGATGTGTGTGT	6650
	TGACGCTT <u>T</u> AACGGGAG	6651
	CTCCCGTT <u>A</u> AAGCGTCA	6652

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Increased stearate stearoyl-ACP desaturase	ACATCATCATCTTCATCAACGATGGCGCTTCGAATGAGTCCGG TGACGCTTCAACGGTAGATATATCCTTCATACACTTTTCATCAATCG AAAAATCTCAGATCTCCTAAATTCGCGA	6653
Helianthus annuus Glu13Term GAG-TAG	TCGCGAATTTAGGAGATCTGAGATTTTTCGATTGATGAAAAGTGTA TGAAGGATATATCTACCGTTGAAGCGTCACCGGACTCATTCGAAG CGCCATCGTTGATGAAGAAGATGATGATGT	6654
	TTCAACGG <u>T</u> AGATATAT	6655
	ATATATCT <u>A</u> CCGTTGAA	6656
Increased stearate stearoyl-ACP desaturase	ATCTTCTTCATCAACGATGGCGCTTCGAATGAGTCCGGTGACGCTT CAACGGGAGATATAGCCTTCATACACTTTTCATCAATCGAAAAATCT CAGATCTCCTAAATTCGCGATGGCTTCC	6657
<i>Helianthus annuus</i> Tyr15Term TAT-TAG	GGAAGCCATCGCGAATTTAGGAGATCTGAGATTTTTCGATTGATGA AAAGTGTATGAAGGCTATATCTCCCGTTGAAGCGTCACCGGACTC ATTCGAAGCGCCATCGTTGATGAAGAAGAT	6658
	GAGATATAGCCTTCATA	6659
	TATGAAGG <u>C</u> TATATCTC	6660
Increased stearate stearoyl-ACP desaturase	AACTCAGCCAGCTTGCCCCCAAACAACAGCGCAGAAAAACCTTCA ACAACAATGGCTCTCTAGCTCAACCCAGTCACCACCTTCCCTTCAA CACGCTCCCTCAACAACTTCTCCTCCAGAT	6661
<i>Linum usitatissimum</i> Lys4Term AAG-TAG	ATCTGGAGGAGAAGTTGTTGAGGGAGCGTGTTGAAGGGAAGGTG GTGACTGGGTTGAGCTAGAGGCCATTGTTGTTGAAGGTTTTTCTG CGCTGTTGTTTGGGGGGCAAGCTGGCTGAGTT	6662
	TGGCTCTCTAGCTCAAC	6663
	GTTGAGCT <u>A</u> GAGAGCCA	6664
Increased stearate stearoyl-ACP desaturase	GCGCAGAAAAACCTTCAACAACAATGGCTCTCAAGCTCAACCCAG TCACCACCTTCCCTTGAACACGCTCCCTCAACAACTTCTCCTCCAG ATCTCCTCGCACCTTTCTCATGGCTGCTTC	6665
Linum usitatissimum Ser13Term TCA-TGA	GAAGCAGCCATGAGAAAGGTGCGAGGAGATCTGGAGGAGAAGTT GTTGAGGGAGCGTGTTCAAGGGAAGGTGGTGACTGGGTTGAGCT TGAGAGCCATTGTTGTAGAGGTTTTTCTGCGC	6666
	CTTCCCTTGAACACGCT	6667
	AGCGTGTT <u>C</u> AAGGGAAG	6668
Increased stearate stearoyl-ACP desaturase	CTCAAGCTCAACCCAGTCACCACCTTCCCTTCAACACGCTCCCTC AACAACTTCTCCTCGGATCTCCTCGCACCTTTCTCATGGCTGCTT CCACTTTCAATTCCACCTCCACCAAGTAAG	6669
Linum usitatissimum Arg23Term AGA-TGA	CTTACTTGGTGGAGGTGGAATTGAAAGTGGAAGCAGCCATGAGAA AGGTGCGAGGAGATCAGGAGGAGAAGTTGTTGAGGGAGCGTGTT GAAGGGAAGG	6670

Phenotype, Gene, Plant & Targeted Alteration	Attering Oligos	SEQID NO:
	TCTCCTCC <u>T</u> GATCTCCT	6671
	AGGAGATC <u>A</u> GGAGGAGA	6672
Increased stearate stearoyl-ACP desaturase	TCCTCCAGATCTCCTCGCACCTTTCTCATGGCTGCTTCCACTTTCA ATTCCACCTCCACCTAGTAAGCATCTCCTCCTCCTCGGAATCTCCG CCGATTTCTTTTAAGCGATTGATCGTAGA	6673
Linum usitatissimum Lys41Term AAG-TAG	TCTACGATCAATCGCTTAAAAGAAATCGGCGGAGATTCCGAGGAG GAGGAGATGCTTACTAGGTGGAGGTGGAATTGAAAGTGGAAGCAG CCATGAGAAAGGTGCGAGGAGATCTGGAGGA	6674
	CCTCCACC <u>T</u> AGTAAGCA	6675
	TGCTTACT <u>A</u> GGTGGAGG	6676
Increased stearate stearoyl-ACP desaturase	ATGGCACTGAAACTTTGCTTTCCACCCCACAAGATGCCTTCCTT	6677
Olea europaea Arg21Term AGA-TGA	CTTTTCCGACCTCCATAGAAGGAGAATGAATAGTTGAAGCCATGAA AACCCTGTGAGATCAGATACGAGCATCGGGGAAGGAAGGCATCTT GTGGGGTGGAAAGCAAAGTTTCAGTGCCAT	6678
	CTCGTATC <u>T</u> GATCTCAC	6679
	GTGAGATC <u>A</u> GATACGAG	6680
Increased stearate stearoyl-ACP desaturase	CCCACAAGATGCCTTCCTTCCCCGATGCTCGTATCAGATCTCACA GGGTTTTCATGGCTTGAACTATTCATTCTCCTTCTATGGAGGTCGG AAAAGTTAAAAAGCCTTTCACGCCTCCACG	6681
Olea europaea Ser29Term TCA-TGA	CGTGGAGGCGTGAAAGGCTTTTTAACTTTTCCGACCTCCATAGAAG GAGAATGAATAGTTCAAGCCATGAAAACCCTGTGAGATCTGATACG AGCATCGGGGAAGGAAGGCATCTTGTGGG	6682
	CATGGCTT <u>G</u> AACTATTC	6683
	GAATAGTT <u>C</u> AAGCCATG	6684
Increased stearate stearoyl-ACP desaturase	GATGCTCGTATCAGATCTCACAGGGTTTTCATGGCTTCAACTATTC ATTCTCCTTCTATGTAGGTCGGAAAAGTTAAAAAGCCTTTCACGCC TCCACGAGAGGTACATGTTCAAGTAACCC	6685
Olea europaea Glu37Term GAG-TAG	GGGTTACTTGAACATGTACCTCTCGTGGAGGCGTGAAAGGCTTTTT AACTTTTCCGACCT <u>A</u> CATAGAAGGAGAATGAATAGTTGAAGCCATG AAAACCCTGTGAGATCTGATACGAGCATC	6686
	CTTCTATG <u>T</u> AGGTCGGA	6687
	TCCGACCT <u>A</u> CATAGAAG	6688

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Phenotype, Gene, Plant & Targeted Alteration	Aftering Oligos	SEQID NO:
Increased stearate stearoyl-ACP desaturase	CGTATCAGATCTCACAGGGTTTTCATGGCTTCAACTATTCATTC	6689
Olea europaea Giy39Term GGA-TGA	AGGAATGGGTTACTTGAACATGTACCTCTCGTGGAGGCGTGAAAG GCTTTTTAACTTTTCAGACCTCCATAGAAGGAGAATGAAT	6690
	TGGAGGTC <u>T</u> GAAAAGTT	6691
	AACTTTTC <u>A</u> GACCTCCA	6692
Increased stearate stearoyl-ACP desaturase	TTCTCGTTTTTGTCGTCCCCTCTGCTCTCTCTCTCTATCAGGCACG GAGAAATGGCACTGTAACTCAGTCCAGTC	6693
Persea americana Lys4Term AAA-TAA	AAGGCGGATAGGAGGCAAGAAATGGAAGCTTCTGAGATTGAAACA TGACTGGACTG	6694
	TGGCACTGTAACTCAGT	6695
	ACTGAGTT <u>A</u> CAGTGCCA	6696
Increased stearate stearoyl-ACP desaturase	CTGCTCTCTCTCTATCAGGCACGGAGAAATGGCACTGAAACTC AGTCCAGTCATGTTTTAATCTCAGAAGCTTCCATTTCTTGCCTCCTA TCCGCCTTCCAATCTCAGATCTCCGAGGG	6697
Persea americana Gln11Term CAA-TAA	CCCTCGGAGATCTGAGATTGGAAGGCGGATAGGAGGCAAGAAAT GGAAGCTTCTGAGATTAAAACATGACTGGACTG	6698
	TCATGTTT <u>T</u> AATCTCAG	6699
	CTGAGATT <u>A</u> AAACATGA	6700
Increased stearate stearoyl-ACP desaturase	TCTCTCTATCAGGCACGGAGAAATGGCACTGAAACTCAGTCCA GTCATGTTTCAATCTTAGAAGCTTCCATTTCTTGCCTCCTATCCGC CTTCCAATCTCAGATCTCCGAGGGTTTTCA	6701
Persea americana Gin13Term CAG-TAG	TGAAAACCCTCGGAGATCTGAGATTGGAAGGCGGATAGGAGGCAA GAAATGGAAGCTTCTAAGATTGAAACATGACTGGACTG	6702
	TTCAATCT <u>T</u> AGAAGCTT	6703
	AAGCTTCT <u>A</u> AGATTGAA	6704
Increased stearate stearoyl-ACP desaturase	CTCTCTATCAGGCACGGAGAAATGGCACTGAAACTCAGTCCAGTC ATGTTTCAATCTCAGTAGCTTCCATTTCTTGCCTCCTATCCGCCTTC CAATCTCAGATCTCCGAGGGTTTTCATGG	6705
Persea americana Lys14Term AAG-TAG	CCATGAAAACCCTCGGAGATCTGAGATTGGAAGGCGGATAGGAGG CAAGAAATGGAAGCTACTGAGATTGAAACATGACTGGACTGAGTTT CAGTGCCATTTCTCCGTGCCTGATAGAGAG	6706

Phenotype, Gene, Plant & Targeted Afteration	Altering Oligos	SEQ ID NO:
	AATCTCAG <u>T</u> AGCTTCCA	6707
	TGGAAGCT <u>A</u> CTGAGATT	6708
Increased stearate stearoyl-ACP desaturase	CCCCGAGATCTCGCTGCCGCTGCTCATGGCGTTCGCGGCGTCCC ACACCGCATCGCCGTAGTCCTGCGGCGCGCGTGGCGCAGAGGAG GAGCAATGGGATGTCGAAGATGGTGGCCATGGCC	6709
Oryza sativa Tyr12Term TAC-TAG	GGCCATGGCCACCATCTTCGACATCCCATTGCTCCTCTCTGCGC CACGCCGCCGCAGGACTACGGCGATGCGGTGTGGGACGCCGCG AACGCCATGAGCAGCGGCAGCGAGATCTCGGGG	6710
	TCGCCGTA <u>G</u> TCCTGCGG	6711
	CCGCAGGACTACGGCGA	6712
Increased stearate stearoyl-ACP desaturase	CTGCTCATGGCGTTCGCGGCGTCCCACACCGCATCGCCGTACTC CTGCGGCGGCGTGGCGT	6713
Oryza sativa Gln19Term CAG-TAG	TGACCCTGTTGATGGTGGAGGCCATGGCCACCATCTTCGACATCC CATTGCTCCTCTACGCCACGCC	6714
	GCGTGGCG <u>T</u> AGAGGAGG	6715
	CCTCCTCTACGCCACGC	6716
Increased stearate stearoyl-ACP desaturase	CCCACACCGCATCGCCGTACTCCTGCGGCGCGTGGCGCAGAG GAGGAGCAATGGGATGTAGAAGATGGTGGCCATCACCA TCAACAGGGTCAAGAACTGCTAAGAAGCCCTACAC	6717
Oryza sativa Ser26Term TCG-TAG	GTGTAGGGCTTCTTAGCAGTCTTGACCCTGTTGATGGTGGAGGCC ATGGCCACCATCTTCTACATCCCATTGCTCCTCTCTGCGCCACG CCGCCGCAGGAGTACGGCGATGCGGTGTGGG	6718
	TGGGATGT <u>A</u> GAAGATGG	6719
	CCATCTTC <u>T</u> ACATCCCA	6720
Increased stearate stearoyl-ACP desaturase	CACACCGCATCGCCGTACTCCTGCGGCGCGCGTGGCGCAGAGGAG GAGCAATGGGATGTCGTAGATGGTGGCCATGGCCTCCACCATCAA CAGGGTCAAGACTGCTAAGAAGCCCTACACTC	6721
Oryza sativa Lys27Term AAG-TAG	GAGTGTAGGGCTTCTTAGCAGTCTTGACCCTGTTGATGGTGGAGG CCATGGCCACCATCTACGACATCCCATTGCTCCTCTCTGCGCCA CGCCGCCGCAGGAGTACGGCGATGCGGTGTG	6722
	GGATGTCG <u>T</u> AGATGGTG	6723
	CACCATCT <u>A</u> CGACATCC	6724

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Increased stearate stearoyl-ACP desaturase	TTCTCTCTAGGTTGAGCGGTTACCAACAGAAGCACTTAGGAGA GAGAAGCAATGGCGTAGAAGCTTCACCACACGGCCTTCAATCCTT CCATGGCGGTTACCTCTTCGGGACTTCCTCG	6725
Simmondsia chinensis Leu3Term TTG-TAG	CGAGGAAGTCCCGAAGAGGTAACCGCCATGGAAGGATTGAAGGC CGTGTGGTGAAGCTTC <u>T</u> ACGCCATTGCTTCTCTCTCAAGTGCTT CTGTTGGTAACCGCTCAACCTAGAGAGAGAA	6726
	AATGGCGT <u>A</u> GAAGCTTC	6727
	GAAGCTTC <u>T</u> ACGCCATT	6728
Increased stearate stearoyl-ACP desaturase	CTCTCTCTAGGTTGAGCGGTTACCAACAGAAGCACTTAGGAGAGAGA	6729
Simmondsia chinensis Lys4Term AAG-TAG	ATCGAGGAAGTCCCGAAGAGGTAACCGCCATGGAAGGATTGAAG GCCGTGTGGTGAAGCTACAACGCCATTGCTTCTCTCTCAAGTG CTTCTGTTGGTAACCGCTCAACCTAGAGAGAG	6730
	TGGCGTTG <u>T</u> AGCTTCAC	6731
	GTGAAGCT <u>A</u> CAACGCCA	6732
Increased stearate stearoyl-ACP desaturase	AAGCAATGGCGTTGAAGCTTCACCACACGGCCTTCAATCCTTCCAT GGCGGTTACCTCTTAGGGACTTCCTCGATCGTATCACCTCAGATC TCACCGCGTTTTCATGGCTTCTTCTACAAT	6733
Simmondsia chinensis Ser19Term TCG-TAG	ATTGTAGAAGAAGCCATGAAAACGCGGTGAGATCTGAGGTGATAC GATCGAGGAAGTCCCTAAGAGGTAACCGCCATGGAAGGATTGAAG GCCGTGTGGTGAAGCTTCAACGCCATTGCTT	6734
]	TACCTCTT <u>A</u> GGGACTTC	6735
	GAAGTCCCTAAGAGGTA	6736
Increased stearate stearoyl-ACP desaturase	GCAATGGCGTTGAAGCTTCACCACACGGCCTTCAATCCTTCCATG GCGGTTACCTCTTCGTGACTTCCTCGATCGTATCACCTCAGATCTC ACCGCGTTTTCATGGCTTCTTCTACAATTG	6737
Simmondsia chinensis Gly20Term GGA-TGA	CAATTGTAGAAGAAGCCATGAAAACGCGGTGAGATCTGAGGTGAT ACGATCGAGGAAGTCACGAAGAGGTAACCGCCATGGAAGGATTGA AGGCCGTGTGGTGAAGCTTCAACGCCATTGC	6738
	CCTCTTCGTGACTTCCT	6739
	AGGAAGTC <u>A</u> CGAAGAGG	6740
Increased stearate stearoyl-ACP desaturase	TGGCTCTGAATCTCAACCCCGTTTCCACACCATTTCAGTGTCGTCG ATTGCCGTCTTTCTGACCTCGTCAAACGCCTTCTCGCAGATCTCCC AAATTCTTCATGGCTTCCACTCTCAGCAG	6741
Spinacia oleracea Ser21Term TCA-TGA	CTGCTGAGAGTGGAAGCCATGAAGAATTTGGGAGATCTGCGAGAA GGCGTTTGACGAGGT <u>C</u> AGAAAGACGGCAATCGACGACACTGAAAT GGTGTGGAAACGGGGTTGAGATTCAGAGCCA	6742

Phenotype, Gene, Plant & Targeted Alteration	Aftering Oligos	SEQ ID NO:
·	GTCTTTCT <u>G</u> ACCTCGTC	6743
	GACGAGGT <u>C</u> AGAAAGAC	6744
Increased stearate stearoyl-ACP desaturase	AATCTCAACCCCGTTTCCACACCATTTCAGTGTCGTCGATTGCCGT CTTTCTCACCTCGTTAAACGCCTTCTCGCAGATCTCCCAAATTCTT CATGGCTTCCACTCTCAGCAGCTCTTCTC	6745
Spinacia oleracea Gln24Term CAA-TAA	GAGAAGAGCTGCTGAGAGTGGAAGCCATGAAGAATTTGGGAGATC TGCGAGAAGGCGTTTAACGAGGTGAGAAAGACGGCAATCGACGA CACTGAAATGGTGTGGAAACGGGGTTGAGATT	6746
	CACCTCGTTAAACGCCT	6747
	AGGCGTTT <u>A</u> ACGAGGTG	6748
Increased stearate stearoyl-ACP desaturase	TCCACACCATTTCAGTGTCGTCGATTGCCGTCTTTCTCACCTCGTC AAACGCCTTCTCGCTGATCTCCCAAATTCTTCATGGCTTCCACTCT CAGCAGCTCTTCTCCTAAGGAAGCGGAAA	6749
Spinacia oleracea Arg29Term AGA-TGA	TTTCCGCTTCCTTAGGAGAGAGGCCTGAGAGTGGAAGCCATGA AGAATTTGGGAGATCAGCGAGAAGGCGTTTGACGAGGTGAGAAAG ACGGCAATCGACGACACTGAAATGGTGTGGA	6750
	CTTCTCGC <u>T</u> GATCTCCC	6751
	GGGAGATC <u>A</u> GCGAGAAG	6752
Increased stearate stearoyl-ACP desaturase	TTTCAGTGTCGATTGCCGTCTTTCTCACCTCGTCAAACGCCTT CTCGCAGATCTCCCTAATTCTTCATGGCTTCCACTCTCAGCAGCTC TTCTCCTAAGGAAGCGGAAAGCCTGAAGA	6753
Spinacia oleracea Lys32Term AAA-TAA	TCTTCAGGCTTTCCGCTTCCTTAGGAGAGAGCTGCTGAGAGTGG AAGCCATGAAGAATTAGGGAGATCTGCGAGAAGGCGTTTGACGAG GTGAGAAAGACGGCAATCGACGACACTGAAA	6754
	GATCTCCC <u>T</u> AATTCTTC	6755
	GAAGAATT <u>A</u> GGGAGATC	6756
Increased stearate stearoyl-ACP desaturase	AAATAGTCGAGGTGAAAAACAGAGCATCAACAATGGCACTGAATAT CAATGGGGTGTCGTGAAAATCTCACAAAATGTTACCATTTCCTTGT TCTTCAGCCAGATCTGAGCGAGTTTTCAT	6757
Solanum tuberosum Leu10Term TTA-TGA	ATGAAAACTCGCTCAGATCTGGCTGAAGAACAAGGAAATGGTAACA TTTTGTGAGATTTTCACGACACCCCATTGATATTCAGTGCCATTGTT GATGCTCTGTTTTTCACCTCGACTATTT	6758
	GGTGTCGT <u>G</u> AAAATCTC	6759
	GAGATITT <u>C</u> ACGACACC	6760

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Phenotype, Gene, Plant & Targeted Alteration	Aftering:Oligos	SEQ ID NO:
Increased stearate stearoyl-ACP desaturase	ATAGTCGAGGTGAAAAACAGAGCATCAACAATGGCACTGAATATCA ATGGGGTGTCGTTATAATCTCACAAAATGTTACCATTTCCTTGTTCT TCAGCCAGATCTGAGCGAGTTTTCATGG	6761
Solanum tuberosum Lys11Term AAA-TAA	CCATGAAAACTCGCTCAGATCTGGCTGAAGAACAAGGAAATGGTA ACATTTTGTGAGATTATAACGACACCCCATTGATATTCAGTGCCATT GTTGATGCTCTGTTTTTCACCTCGACTAT	6762
	TGTCGTTA <u>T</u> AATCTCAC	6763
	GTGAGATT <u>A</u> TAACGACA	6764
Increased stearate stearoyl-ACP desaturase	GTGAAAAACAGAGCATCAACAATGGCACTGAATATCAATGGGGTGT CGTTAAAATCTCAC <u>T</u> AAATGTTACCATTTCCTTGTTCTTCAGCCAGA TCTGAGCGAGTTTTCATGGCTTCAACCA	6765
Solanum tuberosum Lys14Term AAA-TAA	TGGTTGAAGCCATGAAAACTCGCTCAGATCTGGCTGAAGAACAAG GAAATGGTAACATTTAAGGAGATTTTAACGACACCCCATTGATATTC AGTGCCATTGTTGATGCTCTGTTTTTCAC	6766
	AATCTCAC <u>T</u> AAATGTTA	6767
	TAACATTT <u>A</u> GTGAGATT	6768
Increased stearate stearoyl-ACP desaturase	ACAGAGCATCAACAATGGCACTGAATATCAATGGGGTGTCGTTAAA ATCTCACAAAATGTGACCATTTCCTTGTTCTTCAGCCAGATCTGAG CGAGTTTTCATGGCTTCAACCATTCATCG	6769
Solanum tuberosum Leu16Term TTA-TGA	CGATGAATGGTTGAAGCCATGAAAACTCGCTCAGATCTGGCTGAA GAACAAGGAAATGGT <u>C</u> ACATTTTGTGAGATTTTAACGACACCCCAT TGATATTCAGTGCCATTGTTGATGCTCTGT	6770
	CAAAATGT <u>G</u> ACCATTTC	6771
	GAAATGGT <u>C</u> ACATTTTG	6772
Increased stearate stearoyl-ACP desaturase	TGGCTCTGAGGCTGAACCCTAACCCTTCACAGAAGCTCTTTCTCTC TCCTTCTTCATCATCATCATCTTCTTCTTCATCGTTCTCCTC AAATGGCTAGCCTCAGATCTCCAAGGTT	6773
Arachis hypogaea Ser21Term TCA-TGA	AACCTTGGAGATCTGAGGCTAGCCATTTGAGGAAGCGAGAACGAT GAAGAAGAAGAAGATCATGATGAAGAAGGAGAGAAAGAGCTTC TGTGAAGGGTTAGGGTTCAGCCTCAGAGCCA	6774
	TTCATCAT <u>G</u> ATCTTCTT	6775
	AAGAAGAT <u>C</u> ATGATGAA	6776
Increased stearate stearoyl-ACP desaturase	ACCCTAACCCTTCACAGAAGCTCTTTCTCTCTCTTCTTCATCATCA TCTTCTTCTTGATCGTTCTCGCTTCCTCAAATGGCTAGCCTCA GATCTCCAAGGTTCCGCATGGCCTCCAC	6777
Arachis hypogaea Ser26Term TCA-TGA	GTGGAGGCCATGCGGAACCTTGGAGATCTGAGGCTAGCCATTTGA GGAAGCGAGAACGATCAAGAAGAAGAAGATGATGAAGAAGGA GAGAGAAAGAGCTTCTGTGAAGGGTTAGGGT	6778

Phenotype, Gene, Plant & Targeted Alteration	- Altering Oligos	SEQ ID NO:
	TTCTTCTT <u>G</u> ATCGTTCT	6779
	AGAACGAT <u>C</u> AAGAAGAA	6780
Increased stearate stearoyl-ACP desaturase	CTAACCCTTCACAGAAGCTCTTTCTCTCTCCTTCTTCATCATCATCT TCTTCTTCTTCATAGTTCTCGCTTCCTCAAATGGCTAGCCTCAGAT CTCCAAGGTTCCGCATGGCCTCCACCCT	6781
Arachis hypogaea Ser27Term TCG-TAG	AGGGTGGAGGCCATGCGGAACCTTGGAGATCTGAGGCTAGCCAT TTGAGGAAGCGAGAAC <u>T</u> ATGAAGAAGAAGAAGATGATGAAGA AGGAGAGAGAAAGAGCTTCTGTGAAGGGTTAG	6782
	TTCTTCAT <u>A</u> GTTCTCGC	6783
	GCGAGAAC <u>T</u> ATGAAGAA	6784
Increased stearate stearoyl-ACP desaturase	CTTCACAGAAGCTCTTTCTCTCTCTCTTCTTCATCATCATCTTCTTCT TCTTCATCGTTCTAGCTTCCTCAAATGGCTAGCCTCAGATCTCCAA GGTTCCGCATGGCCTCCACCCTCCGCAC	6785
Arachis hypogaea Ser29Term TCG-TAG	GTGCGGAGGGTGGAGGCCATGCGGAACCTTGGAGATCTGAGGCT AGCCATTTGAGGAAGCTAGAACGATGAAGAAGAAGAAGATGATGA TGAAGAAGGAGAGAAAGAGAGCTTCTGTGAAG	6786
	ATCGTTCTAGCTTCCTC	6787
	GAGGAAGC <u>T</u> AGAACGAT	6788
Increased stearate stearoyl-ACP desaturase	AAAGTTAAAAGCCGTCCAAAACCCAAACCAGGAAAGGCAAACGAA AAGAAAAAATGGCTTAGAATTTTAATGCCATCGCCTCGAAATCTCA GAAGCTCCCTTGCTTTGCT	6789
Gossypium hirsutum Leu3Term TTG-TAG	TTTGGTGGAAGAGCAAGCAAGGGAGCTTCTGAGATTTCGAGGCG ATGGCATTAAAATTCTAAGCCATTTTTTCTTTTC	6790
-	AATGGCTT <u>A</u> GAATTTTA	6791
	TAAAATTC <u>T</u> AAGCCATT	6792
Increased stearate stearoyl-ACP desaturase	CCCAAACCAGGAAAGGCAAACGAAAAGAAAAAATGGCTTTGAATTT TAATGCCATCGCCTAGAAATCTCAGAAGCTCCCTTGCTTTGCTCTT CCACCAAAGGCCACCCTTAGATCTCCCAA	6793
Gossypium hirsutum Ser1-Term TCG-TAG	TTGGGAGATCTAAGGGTGGCCTTTGGTGGAAGAGCAAAGCAAGG GAGCTTCTGAGATTTCTAGGCGATGGCATTAAAATTCAAAGCCATT TTTTCTTTTC	6794
	CATCGCCT <u>A</u> GAAATCTC	6795
	GAGATTTC <u>T</u> AGGCGATG	6796

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Phenotype: Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Increased stearate stearoyl-ACP desaturase	CAAACCAGGAAAGGCAAACGAAAAGAAAAAATGGCTTTGAATTTTA ATGCCATCGCCTCGTAATCTCAGAAGCTCCCTTGCTTTGCTCTTCC ACCAAAGGCCACCCTTAGATCTCCCAAGT	6797
Gossypium hirsutum Lys11Term AAA-TAA	ACTTGGGAGATCTAAGGGTGGCCTTTGGTGGAAGAGCAAAGCAAGGGAGCTTCTGAGATTACGAGGCGATGGCATTAAAATTCAAAGCCATTTTTTCTTTTCGTTTGCCTTTCCTGGTTTG	6798
	TCGCCTCGTAATCTCAG	6799
	CTGAGATT <u>A</u> CGAGGCGA	6800
Increased stearate stearoyl-ACP desaturase	AGGAAAGGCAAACGAAAAGAAAAAATGGCTTTGAATTTTAATGCCA TCGCCTCGAAATCTTAGAAGCTCCCTTGCTTTGCT	6801
Gossypium hirsutum Gln13Term CAG-TAG	TGGAAAACTTGGGAGATCTAAGGGTGGCCTTTGGTGGAAGAGCAA AGCAAGGGAGCTTCTAAGATTTCGAGGCGATGGCATTAAAATTCAA AGCCATTTTTCTTTTC	6802
	CGAAATCT <u>T</u> AGAAGCTC	6803
	GAGCTTCT <u>A</u> AGATTTCG	6804

Table 24
Oligonucleotides to produce plants with reduced linolenic acid

Plant & Targeted Alteration  Reduced linolenic acid omega-3 fatty acid desaturase Arabidopsis thaliana Ser4Term TCG-TAG  AATAGAACGACAGAGACTTTTTCCTCTTTTCTTGGGAAGAGGC TCCAATGGCGAGCTAGGTTTTATCAGAATGTGGTTTTAGACCTCTC CCCAGATTCTACCCTAAACACACACCTC GAGGTTGTGTTTAGGGGTAGAATCTGGGGAGAGGTCTAAAACCA CATTCTGATAAAACCTAGCTCGCCATTGGAGCCTCTTCCCAAGAAG AAAAGAGGAAAAAGTCTCTGTCGTTCTATT GGCCGAGCTTGGTTTTAT ATAAAACCAAGCTCGCC	SEQ IB NO: 6805 6806 6807 6808
Reduced linolenic acid omega-3 fatty acid desaturase  Arabidopsis thaliana Ser4Term TCG-TAG AAAAGAGGACAGAGACTTTTTCCTCTTTTCTTCTGGGAAGAGGC TCCAATGGCGAGCTAGGTTTTATCAGAATGTGGTTTTAGACCTCTC CCCAGATTCTACCCTAAACACACACACCTC GAGGTTGTGTTTAGGGTAGAATCTGGGGAGAGGTCTAAAACCA CATTCTGATAAAACCTAGCTCGCCATTGGAGCCTCTTCCCAAGAAG AAAAGAGGAAAAAGTCTCTGTCGTTCTATT GGCCGAGCTTGGTTTTAT	6805 6806 6807 6808
Ser4Term CATTCTGATAAAACCTAGCTCGCCATTGGAGCCTCTTCCCAAGAAG TCG-TAG AAAAGAGGAAAAAGTCTCTGTCGTTCTATT GGCGAGCTTGGTTTTAT	6807 6808
	6808
ATAAAACC <u>A</u> AGCTCGCC	
Reduced linolenic acid omega-3 fatty acid desaturase  ACGACAGAGACTTTTTCCTCTTTTCTTCTGGGAAGAGGCTCCAAT GGCGAGCTCGGTTTGATCAGAACTCTCTCTCCCAG ATTCTACCCTAAACACACAACCTCTTTTGC	6809
15 Arabidopsis thaliana GCAAAAGAGGTTGTGTTTTAGGGTAGAATCTGGGGAGAGGTCTA Leu6Term AAACCACATTCTGATCAAACCGAGCTCGCCATTGGAGCCTCTTCC TTA-TGA CAAGAAGAAAAGAGGAAAAAGTCTCTGTCGT	6810
CTCGGTTT <u>G</u> ATCAGAAT	6811
ATTCTGAT <u>C</u> AAACCGAG	6812
omega-3 fatty acid GAGCTCGGTTTTATGAGACTCTCTCCCCAGATTC desaturase TACCCTAAACACACACACCTCTTTTGCCTC	6813
Ser7Term CTAAAACCACATTCTCATAAAACCGAGCTCGCCATTGGAGCCTCTT TCA-TGA CCCAAGAAGAAAAGAGGAAAAAGTCTCTGT	6814
GGTTTTAT <u>G</u> AGAATGTG	6815
CACATTCT <u>C</u> ATAAAACC	6816
25 omega-3 fatty acid GCTCGGTTTTATCATAATGTGGTTTTAGACCTCTCCCCAGATTCTA desaturase CCCTAAACACACACACCTCTTTTGCCTCTA	6817
GIU8Term GTCTAAAACCACATTATGATAAAACCGAGCTCGCCATTGGAGCCTC GAA-TAA TTCCCAAGAAGAAAAGGAGAAAAAGTCTCT	6818
	6819
ACCACATT <u>A</u> TGATAAAA	6820

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Reduced linolenic acid omega-3 fatty acid desaturase	TCATCATCTTCTTCTGGGGAGAGAGAGAGAGCAAAAGAGCTCT AGCAATGGCGAACTAGGTCTTATCCGAATGTGGCATAAGACCTCT CCCCAGAATCTACACCACACC	6821
Brassica juncea Leu4Term TTG-TAG	GTGGATCTGGGTGTGGTGTAGATTCTGGGGAGAGGTCTTATGCCA CATTCGGATAAGACCTAGTTCGCCATTGCTAGAGCTCTTTTGCTCT CTCTCTCCCCAGAAGAAGAAGATGATGA	6822
	GGCGAACT <u>A</u> GGTCTTAT	6823
	ATAAGACC <u>T</u> AGTTCGCC	6824
Reduced linolenic acid omega-3 fatty acid desaturase	TCTTCTTCTGGGGAGAGAGAGAGAGCAAAAGAGCTCTAGCAA TGGCGAACTTGGTCTGATCCGAATGTGGCATAAGACCTCTCCCCA GAATCTACACCACACC	6825
Brassica juncea Leu6Term TTA-TGA	AGGAAAGTGGATCTGGGTGTGGTGTAGATTCTGGGGAGAGGTCTT ATGCCACATTCGGATCAGACCAAGTTCGCCATTGCTAGAGCTCTTT TGCTCTCTCTCTCCCCAGAAGAAGAAGA	6826
	CTTGGTCTGATCCGAAT	6827
	ATTCGGAT <u>C</u> AGACCAAG	6828
Reduced linolenic acid omega-3 fatty acid desaturase	TTCTTCTGGGGAGAGAGAGAGCAAAAGAGCTCTAGCAATGGCG AACTTGGTCTTATCCTAATGTGGCATAAGACCTCTCCCAGAATCT ACACCACCCCAGATCCACTTTCCTCTCCA	6829
Brassica juncea Glu8Term GAA-TAA	TGGAGAGGAAAGTGGATCTGGGTGTGGTGTAGATTCTGGGGAGA GGTCTTATGCCACATTAGGATAAGACCAAGTTCGCCATTGCTAGAG CTCTTTTGCTCTCTCTCTCCCCAGAAGAA	6830
	TCTTATCC <u>T</u> AATGTGGC	6831
	GCCACATT <u>A</u> GGATAAGA	6832
Reduced linolenic acid omega-3 fatty acid desaturase	CTGGGGAGAGAGAGAGCAAAAGAGCTCTAGCAATGGCGAACTT GGTCTTATCCGAATGAGGCATAAGACCTCTCCCCAGAATCTACAC CACACCCAGATCCACTTTCCTCTCCAACACC	6833
desaurase Brassica juncea Cys9Term TGT-TGA	GGTGTTGGAGAGGAAAGTGGATCTGGGTGTGGTGTAGATTCTGGG GAGAGGTCTTATGCCTCATTCGGATAAGACCAAGTTCGCCATTGCT AGAGCTCTTTTGCTCTCTCTCTCCCCAG	6834
	TCCGAATGAGCATAAG	6835
	CTTATGCC <u>T</u> CATTCGGA	6836
Reduced linolenic acid omega-3 fatty acid desaturase	TATAACAGAATTGCTGAATTCTTGCATTTTTAGCTTCTGGGTTTCAA TGGCTGCTGGTTGAGTATTATCAGAATGTGGTTTAAGGCCTCTCCC AAGAATCTACTCACGACCCAGAATTGGT	6837
Ricinus communis Trp5Term TGG-TGA	ACCAATTCTGGGTCGTGAGTAGATTCTTGGGAGAGGCCTTAAACC ACATTCTGATAATACTCAACCAGCAGCCATTGAAAACCCAGAAGCT AAAAATGCAAGAATTCAGCAATTCTGTTAT	6838

Phenotype, Gene, Plant & Targeted Alteration	Aftering Oligos	SEQ ID
	GCTGGTTG <u>A</u> GTATTATC	6839
	GATAATAC <u>T</u> CAACCAGC	6840
Reduced linolenic acid omega-3 fatty acid desaturase Ricinus communis	AGAATTGCTGAATTCTTGCATTTTTAGCTTCTGGGTTTTCAATGGCT GCTGGTTGGGTATGATCAGAATGTGGTTTAAGGCCTCTCCCAAGA ATCTACTCACGACCCAGAATTGGTTTTAC	6841
Leu7Term TTA-TGA	GTAAAACCAATTCTGGGTCGTGAGTAGATTCTTGGGAGAGGCCTT AAACCACATTCTGATCATCACCAACCAGCAGCCATTGAAAACCCAG AAGCTAAAAATGCAAGAATTCAGCAATTCT	6842
	TTGGGTATGATAGGAAT	6843
	ATTCTGAT <u>C</u> ATACCCAA	6844
Reduced linolenic acid omega-3 fatty acid desaturase	ATTGCTGAATTCTTGCATTTTTAGCTTCTGGGTTTTCAATGGCTGCT GGTTGGGTATTATGAGAATGTGGTTTAAGGCCTCTCCCAAGAATCT ACTCACGACCCAGAATTGGTTTTACATC	6845
Ricinus communis Ser8Term TCA-TGA	GATGTAAAACCAATTCTGGGTCGTGAGTAGATTCTTGGGAGAGGC CTTAAACCACATTCT <u>C</u> ATAATACCCAACCAGCAGCCATTGAAAACC CAGAAGCTAAAAATGCAAGAATTCAGCAAT	6846
	GGTATTAT <u>G</u> AGAATGTG	6847
	CACATTCT <u>C</u> ATAATACC	6848
Reduced linolenic acid omega-3 fatty acid desaturase	TGCTGAATTCTTGCATTTTTAGCTTCTGGGTTTTCAATGGCTGCTG GTTGGGTATTATCATAATGTGGTTTAAGGCCTCTCCCAAGAATCTA CTCACGACCCAGAATTGGTTTTACATCGA	6849
Ricinus communis Glu9Term GAA-TAA	TCGATGTAAAACCAATTCTGGGTCGTGAGTAGATTCTTGGGAGAG GCCTTAAACCACATTATGATAATACCCAACCAGCAGCCATTGAAAA CCCAGAAGCTAAAAATGCAAGAATTCAGCA	6850
	TATTATCA <u>T</u> AATGTGGT	6851
	ACCACATT <u>A</u> TGATAATA	6852
omega-3 fatty acid desaturase	GCAAGTTGGGTTTTATCAGAATGTGGTCTTAGACCACTCCCAAGAA TCTACCCTAAGCCC <u>T</u> GAACTGGGGCAGCCACTTCTGCCTCCTCTC ACATTAAGTTGAGAATTTCACGTACAGATC	6853
Nicotiana tabacum Arg22Term AGA-TGA	GATCTGTACGTGAAATTCTCAACTTAATGTGAGAGGAGGCAGAAGT. GGCTGCCCCAGTTCAGGGCTAGGGTAGATTCTTGGGAGTGGTCT AAGACCACATTCTGATAAAACCCAACTTGC	6854
	CTAAGCCC <u>T</u> GAACTGGG	6855
	CCCAGTTC <u>A</u> GGGCTTAG	6856

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	Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
	Reduced linolenic acid omega-3 fatty acid desaturase	CTCCCAAGAATCTACCCTAAGCCCAGAACTGGGGCAGCCACTTCT GCCTCCTCTCACATTTAGTTGAGAATTTCACGTACAGATCTGAGTG GTTCTGCAATTTCTTTGTCTAATACTAATA	6857
5	Nicotiana tabacum Lys34Term AAG-TAG	TATTAGTATTAGACAAAGAAATTGCAGAACCACTCAGATCTGTACG TGAAATTCTCAACTAAATGTGAGAGGAGGCAGAAGTGGCTGCCCC AGTTCTGGGCTTAGGGTAGATTCTTGGGAG	6858
		CTCACATT <u>T</u> AGTTGAGA TCTCAACT <b>A</b> AATGTGAG	6859 6860
	Reduced linolenic acid	CAAGAATCTACCCTAAGCCCAGAACTGGGGCAGCCACTTCTGCCT	6861
	omega-3 fatty acid desaturase	CCTCTCACATTAAGT <u>A</u> GAGAATTTCACGTACAGATCTGAGTGGTTC TGCAATTTCTTTGTCTAATACTAATAAAGA	
10	Nicotiana tabacum Leu35Term TTG-TAG	TCTTTATTAGTATTAGACAAAGAAATTGCAGAACCACTCAGATCTGT ACGTGAAATTCTCTACTTAATGTGAGAGGGGGGCAGAAGTGGCTGC CCCAGTTCTGGGCTTAGGGTAGATTCTTG	6862
		CATTAAGT <u>A</u> GAGAATTT	6863
		AAATTCTC <u>T</u> ACTTAATG	6864
15 .	Reduced linolenic acid omega-3 fatty acid desaturase	AGAATCTACCCTAAGCCCAGAACTGGGGCAGCCACTTCTGCCTCC TCTCACATTAAGTTGTGAATTTCACGTACAGATCTGAGTGGTTCTG CAATTTCTTTGTCTAATACTAATAAAGAGA	6865
	Nicotiana tabacum Arg36Term AGA-TGA	TCTCTTTATTAGTATTAGACAAAGAAATTGCAGAACCACTCAGATCT GTACGTGAAATTCACAACTTAATGTGAGAGGAGGCAGAAGTGGCT GCCCCAGTTCTGGGCTTAGGGTAGATTCT	6866
		TTAAGTTG <u>T</u> GAATTTCA	6867
		TGAAATTC <u>A</u> CAACTTAA	6868
20	Reduced linolenic acid omega-3 fatty acid desaturase	GCGAGTTGGGTTTTATCAGAATGTGGTCTGAGGCCACTCCCGAGG GTCTATCCTAAGCCATGAACTGGCCACCCTTTGTTGAATTCCAATC CCACAAAGCTGAGATTTTCAAGAACAGATC	6869
	Sesamum indicum Arg22Term AGA-TGA	GATCTGTTCTTGAAAATCTCAGCTTTGTGGGATTGGAATTCAACAA AGGGTGGCCAGTTCATGGCTTAGGATAGACCCTCGGGAGTGGCC TCAGACCACATTCTGATAAAACCCAACTCGC	6870
		CTAAGCCA <u>T</u> GAACTGGC	6871
		GCCAGTTC <u>A</u> TGGCTTAG	6872
25	Reduced linolenic acid omega-3 fatty acid desaturase	CAGAATGTGGTCTGAGGCCACTCCCGAGGGTCTATCCTAAGCCAA GAACTGGCCACCCTTAGTTGAATTCCAATCCCACAAAGCTGAGATT TTCAAGAACAGATCTTGGAAATGGTTCTTC	6873
	Sesamum indicum Leu27Term	GAAGAACCATTTCCAAGATCTGTTCTTGAAAATCTCAGCTTTGTGG GATTGGAATTCAAC <u>T</u> AAGGGTGGCCAGTTCTTGGCTTAGGATAGA	6874
30	TTG-TAG	CCCTCGGGAGTGGCCTCAGACCACATTCTG	

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQID No:
	CCACCCTT <u>A</u> GTTGAATT	6875
	AATTCAAC <u>T</u> AAGGGTGG	6876
Reduced linolenic acid	AATGTGGTCTGAGGCCACTCCCGAGGGTCTATCCTAAGCCAAGAA	6877
omega-3 fatty acid	CTGGCCACCCTTTGTAGAATTCCAATCCCACAAAGCTGAGATTTTC	
desaturase	AAGAACAGATCTTGGAAATGGTTCTTCATT	
Sesamum indicum	AATGAAGAACCATTTCCAAGATCTGTTCTTGAAAAATCTCAGCTTTGT	6878
Leu28Term	GGGATTGGAATTCTACAAAGGGTGGCCAGTTCTTGGCTTAGGATA	·
TTG-TAG	GACCCTCGGGAGTGGCCTCAGACCACATT	
	CCCTTTGTAGAATTCCA	6879
	TGGAATTC <u>T</u> ACAAAGGG	6880
Reduced linolenic acid	CTCCCGAGGGTCTATCCTAAGCCAAGAACTGGCCACCCTTTGTTG	6881
omega-3 fatty acid	AATTCCAATCCCACA <u>T</u> AGCTGAGATTTTCAAGAACAGATCTTGGAA	
desaturase	ATGGTTCTTCATTCTGTTTGTCGAGTGGGA	
Sesamum indicum	TCCCACTCGACAAACAGAATGAAGAACCATTTCCAAGATCTGTTCT	6882
Lys34Term AAG-TAG	TGAAAATCTCAGCT <u>A</u> TGTGGGATTGGAATTCAACAAAGGGTGGCC	
AAG-TAG	AGTTCTTGGCTTAGGATAGACCCTCGGGAG	
	ATCCCACA <u>T</u> AGCTGAGA	6883
	TCTCAGCT <u>A</u> TGTGGGAT	6884
Reduced linolenic acid	CATCAGAGCGCCGATACCTAAGCATTGCTGGGTTAAGAATCCATG	-6885
omega-3 fatty acid	GAAGTCTATGAGTTAGGTCGTCAGAGAGCTAGCCATCGTGTTCGC	
desaturase	ACTAGCTGCTGGAGCTGCTTACCTCAACAAT	
Brassica napus	ATTGTTGAGGTAAGCAGCTCCAGCAGCTAGTGCGAACACGATGGC	6886
Tyr3Term TAC-TAG	TAGCTCTCTGACGACCTAACTCATAGACTTCCATGGATTCTTAACC	
IAG-IAG	CAGCAATGCTTAGGTATCGCCGCTCTGATG ATGAGTTAGGTCGTCAG	
		6887
	CTGACGAC <u>C</u> TAACTCAT	6888
Reduced linolenic acid	GCGGCGATACCTAAGCATTGCTGGGTTAAGAATCCATGGAAGTCT	6889
omega-3 fatty acid	ATGAGTTACGTCGTGTGAGAGCTAGCCATCGTGTTCGCACTAGCT	
desaturase	GCTGGAGCTGCTTACCTCAACAATTGGCTTG	
Brassica napus Arg6Term	CAAGCCAATTGTTGAGGTAAGCAGCTCCAGCAGCTAGTGCGAACA	6890
AGA-TGA	CGATGGCTAGCTCTCAGACGACGTAACTCATAGACTTCCATGGAT	
,	TCTTAACCCAGCAATGCTTAGGTATCGCCGC ACGTCGTCTGAGAGCTA	0004
Ł		6891
	TAGCTCTCAGACGACGT	6892

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Phenotype, Gene, Plant & Targeted Afteration	Attering Oligos	SEQID No:
Reduced linolenic acid omega-3 fatty acid desaturase	GCGATACCTAAGCATTGCTGGGTTAAGAATCCATGGAAGTCTATGA GTTACGTCGTCAGATAGCTAGCCATCGTGTTCGCACTAGCTGCTG GAGCTGCTTACCTCAACAATTGGCTTGTTT	6893
Brassica napus Glu7Term GAG-TAG	AAACAAGCCAATTGTTGAGGTAAGCAGCTCCAGCAGCTAGTGCGA ACACGATGGCTAGCTATCTGACGACGTAACTCATAGACTTCCATG GATTCTTAACCCAGCAATGCTTAGGTATCGC	6894
	TCGTCAGATAGCTAGCC	6895
	GGCTAGCT <u>A</u> TCTGACGA	6896
Reduced linolenic acid omega-3 fatty acid desaturase	CCATGGAAGTCTATGAGTTACGTCGTCAGAGAGCTAGCCATCGTG TTCGCACTAGCTGCTTGAGCTGCTTACCTCAACAATTGGCTTGTTT GGCCTCTCTATTGGATTGCTCAAGGAACCA	6897
Brassica napus Gly17Term GGA-TGA	TGGTTCCTTGAGCAATCCAATAGAGAGGCCAAACAAGCCAATTGTT GAGGTAAGCAGCTCAAGCAGCTAGTGCGAACACGATGGCTAGCT CTCTGACGACGTAACTCATAGACTTCCATGG	6898
	TAGCTGCTTGAGCTGCT	6899
	AGCAGCTC <u>A</u> AGCAGCTA	6900
Reduced linolenic acid omega-3 fatty acid desaturase Solanum tuberosum Arg22Term AGA-TGA	GCAAGTTGGGTTCTATCAGAATGTGGTCTTAGACCACTACCAAGAA TATACCCAAAGCCCTGAATAGGGTCTTCTTCCGTTTGCGCCACCAA TTTAAATCTGAGAAGAATTTCACCTTCAC	6901
	GTGAAGGTGAAATTCTTCTCAGATTTAAATTGGTGGCGCAAACGGA AGAAGACCCTATTCAGGGCTTTGGGTATATTCTTGGTAGTGGTCTA AGACCACATTCTGATAGAACCCAACTTGC	6902
	CAAAGCCC <u>T</u> GAATAGGG	6903
	CCCTATTCAGGGCTTTG	6904
Reduced linolenic acid omega-3 fatty acid desaturase	TGGTCTTAGACCACTACCAAGAATATACCCAAAGCCCAGAATAGG GTCTTCTTCCGTTTGAGCCACCAATTTAAATCTGAGAAGAATTTCA CCTTCACCTATACGAACAGATCGGAATTGT	6905
Solanum tuberosum Cys29Term TGC-TGA	ACAATTCCGATCTGTTCGTATAGGTGAAGGTGAAATTCTTCTCAGA TTTAAATTGGTGGCTCAAACGGAAGAAGACCCTATTCTGGGCTTTG GGTATATTCTTGGTAGTGGTCTAAGACCA	6906
	TCCGTTTG <u>A</u> GCCACCAA	6907
	TTGGTGGC <u>T</u> CAAACGGA	6908
Reduced linolenic acid omega-3 fatty acid desaturase	CACTACCAAGAATATACCCAAAGCCCAGAATAGGGTCTTCTTCCGT TTGCGCCACCAATTGAAATCTGAGAAGAATTTCACCTTCACCTATA CGAACAGATCGGAATTGTTGGGCATTGAG	6909
Solanum tuberosum Leu33Term TTA-TGA	CTCAATGCCCAACAATTCCGATCTGTTCGTATAGGTGAAGGTGAAA TTCTTCTCAGATTT <u>C</u> AATTGGTGGCGCAAACGGAAGAAGACCCTAT TCTGGGCTTTGGGTATATTCTTGGTAGTG	6910

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID
	CACCAATT <u>G</u> AAATCTGA	6911
	TCAGATTT <u>C</u> AATTGGTG	6912
Reduced linolenic acid omega-3 fatty acid desaturase	AGAATATACCCAAAGCCCAGAATAGGGTCTTCTTCCGTTTGCGCCA CCAATTTAAATCTGTGAAGAATTTCACCTTCACCTATACGAACAGAT CGGAATTGTTGGGCATTGAGGGTAAGTG	6913
Solanum tuberosum Arg36Term AGA-TGA	CACTTACCCTCAATGCCCAACAATTCCGATCTGTTCGTATAGGTGA AGGTGAAATTCTTCACAGATTTAAATTGGTGGCGCAAACGGAAGAA GACCCTATTCTGGGCTTTGGGTATATTCT	6914
İ	TAAATCTG <u>T</u> GAAGAATT	6915
	AATTCTTCACAGATTTA	6916
Reduced linolenic acid omega-3 fatty acid desaturase	CTCTTTATTATCCTCCTCTTCTTTGTTTTTTTTGAGTTCTGAGTCACC TATGGCAAGTTGAGTGATTTCAGAATGTGGGCTAAGGCCACTTCC AAGAATCTATGCCAGGCCCAGAAGTGGA	6917
Petroselinum crispum Trp4Term TGG-TGA	TCCACTTCTGGGCCTGGCATAGATTCTTGGAAGTGGCCTTAGCCC ACATTCTGAAATCACTCAACTTGCCATAGGTGACTCAGAACTCAAA AAAAACAAAGAAGAGGGGGGATAATAAAGAG	6918
	GCAAGTTG <u>A</u> GTGATTTC	6919
	GAAATCAC <u>T</u> CAACTTGC	6920
Reduced linolenic acid omega-3 fatty acid desaturase	TATCCTCCTCTTCTTTGTTTTTTTGAGTTCTGAGTCACCTATGGCA AGTTGGGTGATTTGAGAATGTGGGCTAAGGCCACTTCCAAGAATC TATGCCAGGCCCAGAAGTGGAGCTTCATG	6921
Petroselinum crispum Ser7Term TCA-TGA	CATGAAGCTCCACTTCTGGGCCTGGCATAGATTCTTGGAAGTGGC CTTAGCCCACATTCTCAAATCACCCAACTTGCCATAGGTGACTCAG AACTCAAAAAAAACAAGAAGAAGAGGAGGATA	6922
	GGTGATTT <u>G</u> AGAATGTG	6923
	CACATTCT <u>C</u> AAATCACC	6924
omega-3 fatty acid desaturase	TCCTCCTCTTCTTTGTTTTTTTGAGTTCTGAGTCACCTATGGCAAG TTGGGTGATTTCATAATGTGGGCTAAGGCCACTTCCAAGAATCTAT GCCAGGCCCAGAAGTGGAGCTTCATGTT	6925
Petroselinum crispum Glu8Term GAA-TAA	AACATGAAGCTCCACTTCTGGGCCTGGCATAGATTCTTGGAAGTG GCCTTAGCCCACATTATGAAATCACCCAACTTGCCATAGGTGACTC AGAACTCAAAAAAAACAAAGAAGAGGAGGA	6926
	TGATTTCA <u>T</u> AATGTGGG	6927
	CCCACATT <u>A</u> TGAAATCA	6928

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Phenotype, Gene. Plant & Targeted Alteration	Altering Oligos	SEQID NO:
Reduced linolenic acid omega-3 fatty acid desaturase	CTCTTCTTTGTTTTTTTGAGTTCTGAGTCACCTATGGCAAGTTGGG TGATTTCAGAATGAGGGCCTAAGGCCACTTCCAAGAATCTATGCCA GGCCCAGAAGTGGAGCTTCATGTTTCAAC	6929
Petroselinum crispum Cys9Term TGT-TGA	GTTGAAACATGAAGCTCCACTTCTGGGCCTGGCATAGATTCTTGGA AGTGGCCTTAGCCC <u>T</u> CATTCTGAAATCACCCAACTTGCCATAGGTG ACTCAGAACTCAAAAAAAAAA	6930
	TCAGAATGAGGGCTAAG	6931
Reduced linolenic acid	CTTAGCCC <u>T</u> CATTCTGA  IATGAAGCAGCAACAGTACAAAGACACCCCAATTCTAAATGGCGTTA	6932 6933
omega-3 fatty acid desaturase	ATGGTTTTCATGCTTAAGAAGAAGAAGAAGAAGAAGAAGATTTCGACTT AAGCAATCCTCCTCCATTCAATATTGGTC	0933
Vemicia fordii Lys21Term AAA-TAA	GACCAATATTGAATGGAGGAGGATTGCTTAAGTCGAAATCCTCTTC TTCTTCTTCTTAAGCATGAAAACCATTAACGCCATTTAGAATTG GGGTGTCTTTGTACTGTTGCTGCTTCAT	6934
	TTCATGCT <u>T</u> AAGAAGAA	6935
	TTCTTCTT <u>A</u> AGCATGAA	6936
Reduced linolenic acid omega-3 fatty acid desaturase	AAGCAGCAACAGTACAAAGACACCCCAATTCTAAATGGCGTTAATG GTTTTCATGCTAAA <u>T</u> AAGAAGAAGAAGAAGAGGATTTCGACTTAAG CAATCCTCCTCCATTCAATATTGGTCAGA	6937
Vemicia fordii Glu22Term GAA-TAA	TCTGACCAATATTGAATGGAGGAGGATTGCTTAAGTCGAAATCCTC TTCTTCTTCTTATTTAGCATGAAAACCATTAACGCCATTTAGAA TTGGGGTGTCTTTGTACTGTTGCTGCTT	6938
	ATGCTAAA <u>T</u> AAGAAGAA	6939
	TTCTTCTT <u>A</u> TTTAGCAT	6940
Reduced linolenic acid omega-3 fatty acid desaturase	CAGCAACAGTACAAAGACACCCCAATTCTAAATGGCGTTAATGGTT TTCATGCTAAAGAATAAGAAGAAGAAGAGGGATTTCGACTTAAGCAA TCCTCCTCCATTCAATATTGGTCAGATCC	6941
Vemicia fordii Glu23Term GAA-TAA	GGATCTGACCAATATTGAATGGAGGAGGATTGCTTAAGTCGAAATC CTCTTCTTCTTATTCTTTAGCATGAAAACCATTAACGCCATTTA GAATTGGGGTGTCTTTGTACTGTTGCTG	6942
	CTAAAGAA <u>T</u> AAGAAGAA	6943
	TTCTTCTT <u>A</u> TTCTTTAG	6944
Reduced linolenic acid omega-3 fatty acid desaturase	CAGCAACAGTACAAAGACACCCCAATTCTAAATGGCGTTAATGGTT TTCATGCTAAAGAA <u>T</u> AAGAAGAAGAAGAGGATTTCGACTTAAGCAA TCCTCCTCCATTCAATATTGGTCAGATCC	6945
<i>Vemicia fordii</i> Glu24Term GAA-TAA	GGATCTGACCAATATTGAATGGAGGAGGATTGCTTAAGTCGAAATC CTCTTCTTCTTATTCTTTAGCATGAAAACCATTAACGCCATTTA GAATTGGGGTGTCTTTGTACTGTTGCTG	6946

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ I
	CTAAAGAA <u>T</u> AAGAAGAA	6947
	TTCTTCTTATTCTTTAG	6948
Reduced linolenic acid omega-3 fatty acid desaturase	GGTCCAAGCACAGCCTCTACAACATGTTGGTAATGGTGCAGGGAA AGAAGATCAAGCTTAGTTTGATCCAAGTGCTCCACCACCCTTCAAG ATTGCAAATATCAGAGCAGCAATTCCAAAA	6949
<i>Glycine max</i> Tyr21Term TAT-TAG	TTTTGGAATTGCTGCTCTGATATTTGCAATCTTGAAGGGTGGTGGA GCACTTGGATCAAACTAAGCTTGATCTTCTTTCCCTGCACCATTAC CAACATGTTGTAGAGGCTGTGCTTGGACC	6950
	CAAGCTTA <u>G</u> TTTGATCC	6951
	GGATCAAA <u>C</u> TAAGCTTG	6952
Reduced linolenic acid omega-3 fatty acid desaturase	GGTAATGGTGCAGGGAAAGAAGATCAAGCTTATTTTGATCCAAGTG CTCCACCACCCTTCTAGATTGCAAATATCAGAGCAGCAATTCCAAA ACATTGCTGGGAGAAGAACACATTGAGAT	6953
Glycine max Lys31Term AAG-TAG	ATCTCAATGTGTTCTCCCCAGCAATGTTTTGGAATTGCTGCTCT GATATTTGCAATCTAGAAGGGTGGTGGAGCACTTGGATCAAAATAA GCTTGATCTTCCCTGCACCATTACC	6954
	CACCCTTC <u>T</u> AGATTGCA	695
	TGCAATCT <u>A</u> GAAGGGTG	695
Reduced linolenic acid omega-3 fatty acid desaturase	AAAGAAGATCAAGCTTATTTTGATCCAAGTGCTCCACCACCCTTCA AGATTGCAAATATCTGAGCAGCAATTCCAAAACATTGCTGGGAGAA GAACACATTGAGATCTCTGAGTTATGTTC	695
Glycine max Arg36Term AGA-TGA	GAACATAACTCAGAGATCTCAATGTGTTCTTCTCCCAGCAATGTTTT GGAATTGCTGCTCAGATATTTGCAATCTTGAAGGGTGGTGGAGCA CTTGGATCAAAATAAGCTTGATCTTCTTT	695
	CAAATATC <u>T</u> GAGCAGCA	6959
	TGCTGCTC <u>A</u> GATATTTG	696
Reduced linolenic acid omega-3 fatty acid desaturase Glycine max Leu41Term	TATTTTGATCCAAGTGCTCCACCACCCTTCAAGATTGCAAATATCA GAGCAGCAATTCCA <u>T</u> AACATTGCTGGGAGAAGAACACATTGAGATC TCTGAGTTATGTTCTGAGGGATGTGTTGG	6961
	CCAACACATCCCTCAGAACATAACTCAGAGATCTCAATGTGTTCTT CTCCCAGCAATGTTATGGAATTGCTGCTCTGATATTTGCAATCTTG AAGGGTGGTGGAGCACTTGGATCAAAATA	6962
	CAATTCCA <u>T</u> AACATTGC	6963
	GCAATGTT <u>A</u> TGGAATTG	6964

Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Reduced linolenic acid omega-3 fatty acid desaturase	CATCCACCGCACCCGCACCCGCTGACGGCGCAATGGC CCGGCTCGTGCTCTCCTAGTGCTCGGGCCTCGCGCCCGCC	6965
Zea mays Glu8Term GAG-TAG	GCGCCGCAATGGCGCCCGGCCGGCGGCGGACGG GCGCGAGGCCCGAGCACT <u>A</u> GGAGAGCACGAGCCGGGCCATTGC CGCCGTCAGCGGGCGGGTGCGGGTGGATG	6966
	TGCTCTCCTAGTGCTCG	6967
	CGAGCACT <u>A</u> GGAGAGCA	6968
Reduced linolenic acid omega-3 fatty acid desaturase	ACCCGCACCCGCACCCGCCGCTGACGGCGGCAATGGCCCGGCTCGTGCTCTCCGAGTGATCGGGCCTCGCGCCCGTCCGCCCCTCGCGCCCGCC	6969
Zea mays Cys9Term TGC-TGA	TGACCGCGCCGCAATGGCGCCCCGGCCGGCGCGCGCGCGC	6970
	TCCGAGTG <u>A</u> TCGGGCCT	6971
	AGGCCCGA <u>T</u> CACTCGGA	6972
Reduced linolenic acid omega-3 fatty acid desaturase	CCGCACCCGCACCCGCCCGCTGACGGCGGCAATGGCCCGGCTCGTGCTGCTGCTCCGCGCCGCCGCCGCCGC	6973
Zea mays Ser10Term TCG-TAG	GGTGACCGCGCAATGGCGCCCCGGCCGCGCGCAGGCGGC GGACGGCGCGAGGCCCTAGCACTCGGAGAGCACGAGCCGGGC CATTGCCGCCGTCAGCGGGGCGGG	6974
	CGAGTGCT <u>A</u> GGGCCTCG	6975
	CGAGGCCC <u>T</u> AGCACTCG	6976
Reduced linolenic acid omega-3 fatty acid desaturase	GCTCGGGCCTCGCGCCGCCGCGGGGCCGGGGCCGCCGCCG	6977
Zea mays Ser29Term TCA-TGA	TCGCGGTGGATGGACGCGGACGCGGGCGCGCGCGCGCGCG	6978
	GGCGCGGT <u>G</u> ACCCCCCG	6979
	CGGGGGT <u>C</u> ACCGCGCC	6980
Reduced linolenic acid omega-3 fatty acid desaturase	CCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCCC	6981
<i>Triticum aestivum</i> Glu8Term GAG-TAG	GCTTGGCGCGTCGAACTCGGAGCGGTGGTCCTCGGTGGCCTTG CAGCTCGCCTCCTGCTACGGCCTCATTGCGGGGGCCATGGCCGC GGATGGATCTGTGCGTGTGCGTGGGGGAGGGGG	6982

Phenotype, Gene, Plant & Targeted Alteration	Aftering Oligos	SEQ ID
	TGAGGCCG <u>T</u> AGCAGGAG	6983
	CTCCTGCTACGGCCTCA	6984
Reduced linolenic acid omega-3 fatty acid desaturase	CCTCCCCACGCACACGCACAGATCCATCCGCGGCCATGGCCCC CGCAATGAGGCCGGAGTAGGAGGCGAGCTGCAAGGCCACCGAG GACCACCGCTCCGAGTTCGACGCCGCCAAGCCGC	6985
Triticum aestivum Gln9Term CAG-TAG	GCGGCTTGGCGGCGTCGAACTCGGAGCGGTGGTCCTCGGTGGC CTTGCAGCTCGCCTCCTACTCCGGCCTCATTGCGGGGGCCATGG CCGCGGATGGATCTGTGCGTGGGGGGGAGG	6986
	GGCCGGAG <u>T</u> AGGAGGCG	6987
	CGCCTCCTACTCCGGCC	6988
Reduced linolenic acid omega-3 fatty acid desaturase	CCCCACGCACACGCACAGATCCATCCGCGGCCATGGCCCCCGC AATGAGGCCGGAGCAGTAGGCGAGCTGCAAGGCCACCGAGGACC ACCGCTCCGAGTTCGACGCCGCCAAGCCGCCGC	6989
Triticum aestivum Glu10Term GAG-TAG	GCGGCGGCTTGGCGCGTCGAACTCGGAGCGGTGGTCCTCGGT GGCCTTGCAGCTCGCCTACTGCTCCGGCCTCATTGCGGGGGCCA TGGCCGCGGATGGATCTGTGCGTGTGCGTGGGGG	6990
	CGGAGCAG <u>T</u> AGGCGAGC	6991
	GCTCGCCT <u>A</u> CTGCTCCG	6992
Reduced linolenic acid omega-3 fatty acid desaturase	ACGCACAGATCCATCCGCGGCCATGGCCCCGCAATGAGGCCGG AGCAGGAGGCGAGCTGAAAGGCCACCGAGGACCACCGCTCCGA GTTCGACGCCGCCAAGCCGCCCCCTTCCGCATC	6993
Triticum aestivum Cys13Term TGC-TGA	GATGCGAAGGGCGGCGGCTTGGCGGCGCGGGGGGTGGTCCTCGGGCGCCTCATT TGGTCCTCGGTGGCCTTTCAGCTCGCCTCCTGCTCCGGCCTCATT GCGGGGGCCATGGCCGCGGATGGATCTGTGCGT	6994
	GCGAGCTG <u>A</u> AAGGCCAC	6995
	GTGGCCTTTCAGCTCGC	6996
Reduced linolenic acid omega-3 fatty acid desaturase	CTTCACAAATCACAAATCGGAATCAGATCCACCACGACACCCCGG CGGCAATGGCGGCGTAGGCGACCCAGGAGGCCGACTGCAAGGC TTCCGAGGACGCCCGTCTCTTCTTCGACGCCGC	6997
<i>Oryza sativa</i> Ser4Term TCG-TAG	GCGCCTCGAAGAAGAGACGGCCTCCTCGGAAGCCTTGCAGTC GGCCTCCTGGGTCGCCTACGCCGCCATTGCCGCCGGGGTGTCGT GGTGGATCTGATTCCGATTTGTGAAG	6998
	GGCGGCGT <u>A</u> GGCGACCC	6999
	GGGTCGCC <u>T</u> ACGCCGCC	7000

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Phenotype, Gene, Plant & Targeted Alteration	Altering Oligos	SEQ ID NO:
Reduced linolenic acid omega-3 fatty acid desaturase	ATCACAAATCGGAATCAGATCCACCACGACACCCCGGCGCAATG GCGGCGTCGGCGACCTAGGAGGCCGACTGCAAGGCTTCCGAGG ACGCCCGTCTCTTCTTCGACGCCGCCAAGCCCC	
Oryza sativa Gin7Term CAG-TAG	GGGGCTTGGCGGCGTCGAAGAAGAGACGGGCGTCCTCGGAAGC CTTGCAGTCGGCCTCCTAGGTCGCCGACGCCGCCATTGCCGCCG GGGTGTCGTGGTGGATCTGATTCCGATTTGTGAT	7002
	CGGCGACC <u>T</u> AGGAGGCC	7003
	GGCCTCCT <u>A</u> GGTCGCCG	7004
Reduced linolenic acid omega-3 fatty acid desaturase	ACAAATCGGAATCAGATCCACCACGACACCCCGGCGCAATGGC GGCGTCGGCGACCCAGTAGGCCGACTGCAAGGCTTCCGAGGACG CCCGTCTCTTCTTCGACGCCGCCAAGCCCCCGC	
Oryza sativa Glu8Term GAG-TAG	GCGGGGCTTGGCGCCTCGAAGAAGAGACGGCCTCCTCGGA AGCCTTGCAGTCGGCCTACTGGGTCGCCGACGCCGCCATTGCCG CCGGGGTGTCGTGGTGGATCTGATTCCGATTTGT	7006
	CGACCCAG <u>T</u> AGGCCGAC	7007
	GTCGGCCT <u>A</u> CTGGGTCG	7008
Reduced linolenic acid omega-3 fatty acid desaturase	TCAGATCCACCACGACACCCCGGCGCGAATGGCGGCGTCGGCGA CCCAGGAGGCCGACTGAAAGGCTTCCGAGGACGCCCGTCTCTTC TTCGACGCCGCCAAGCCCCCGCCCTTCCGCATC	7009
Oryza sativa Cys10Term TGC-TGA	GATGCGGAAGGGCGGGGGCTTGGCGGCGTCGAAGAAGAGACGG GCGTCCTCGGAAGCCTTTCAGTCGGCCTCCTGGGTCGCCGACGC CGCCATTGCCGCCGGGTGTCGTGGTGGATCTGA	7010
	GCCGACTG <u>A</u> AAGGCTTC	7011
	GAAGCCTT <u>T</u> CAGTCGGC	7012

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#### WHAT IS CLAIMED IS:

- 1. An oligonucleotide for targeted alteration of genetic sequence, comprising a single-stranded oligonucleotide having a DNA domain, said DNA domain having at least one mismatch with respect to the genetic sequence to be altered, and further comprising chemical modifications of the oligonucleotide, said chemical modifications selected from the group consisting of an o-methyl modification, an LNA modification including LNA derivatives and analogs, two or more phosphorothioate linkages on a terminus, and a combination of any two or more of these modifications.
- 2. The oligonucleotide according to claim one that comprises two or more phosphorothicate linkages on at least the 3' terminus.
  - 3. The oligonucleotide according to claim one that comprises a 2'-O-methyl analog.
- 4. The oligonucleotide according to claim one that comprises an LNA nucleotide, including an LNA derivative or analog.
- 5. The oligonucleotide according to claim one that comprises a combination of at least two modifications selected from the group of a phosphorothioate linkage, a 2'-O-methyl analog, a locked nucleotide analog and a ribonucleotide.
- 6. The oligonucleotide according to any one of claims 1 to 5 that comprises at least one unmodified ribonucleotide.
- 7. The oligonucleotide according to any one of claims 1 to 6, wherein the sequence of said oligonucleotide is selected from the group consisting of SEQ ID NOS: 4341-7012.
- 8. A method of targeted alteration of genetic material, comprising combining the target genetic material with an oligonucleotide according to any one of claims 1 to 7 in the presence of purified proteins.

- 9. A method of targeted alteration of genetic material, comprising administering to a cell extract an oligonucleotide of any one of claims 1 to 7.
- 10. A method of targeted alteration of genetic material, comprising administering to a cell an oligonucleotide of any one of claims 1 to 7.
- 11. A method of targeted alteration of genetic sequence in callus, comprising administering to the callus an oligonucleotide of any one of claims 1 to 7.
- 12. A method of targeted alteration of genetic sequence, comprising combining target genetic material with an oligonucleotide according to any one of claims 1 to 7, said target genetic material being a non-transcribed DNA strand of a duplex DNA.
  - 13. The genetic material obtained by any one of the methods of claim 8, 9 or claim 10.
  - 14. A cell comprising the genetic material of claim 13.
  - 15. A plant organism comprising the cell according to claim 14.
  - 16. A plant or plant part produced by the method of claim 11.
- 17. A method of determining whether an oligonucleotide is optimized for targeted alteration of a genetic sequence, which comprises:
- (a) comparing the efficiency of alteration of a targeted genetic sequence by an oligonucleotide of any one of claims 1 to 7 with the efficiency of alteration of the same targeted genetic sequence by a second oligonucleotide, said second oligonucleotide selected from the group of an oligonucleotide that lacks the mismatch, a fully modified phosphorothiolated oligonucleotide, a fully modified 2'-O-methylated oligonucleotide and a chimeric double-stranded double hairpin containing RNA and DNA nucleotides.

- 18. The method of claim 17 in which the alteration is produced in a plant cell extract.
- 19. The method of claim 17 in which the alteration is produced in a cell.
- 20. A kit comprising the oligonucleotide according to any one of claims 1 to 7 and a second oligonucleotide selected from the group of an oligonucleotide that lacks the mismatch, a fully modified phosphorothiolated oligonucleotide, a fully modified 2-O-methylated oligonucleotide and a chimeric double stranded double hairpin containing RNA and DNA nucleotides.

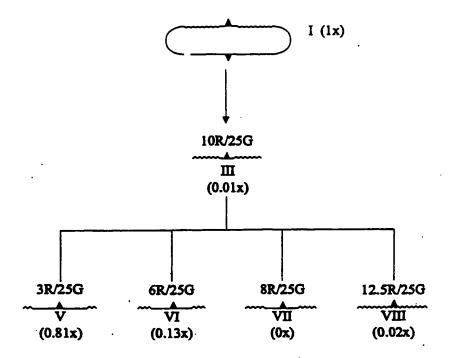


Figure 1A

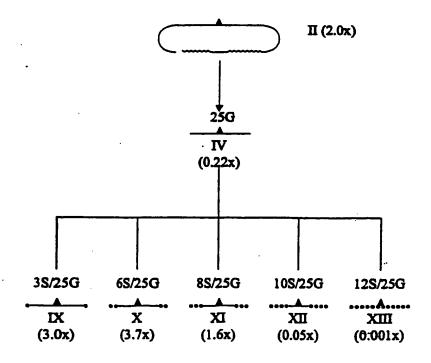


Figure 1B SUBSTITUTE SHEET (RULE 26)

Plasmids, DNA targets and chimeric oligonucleotides

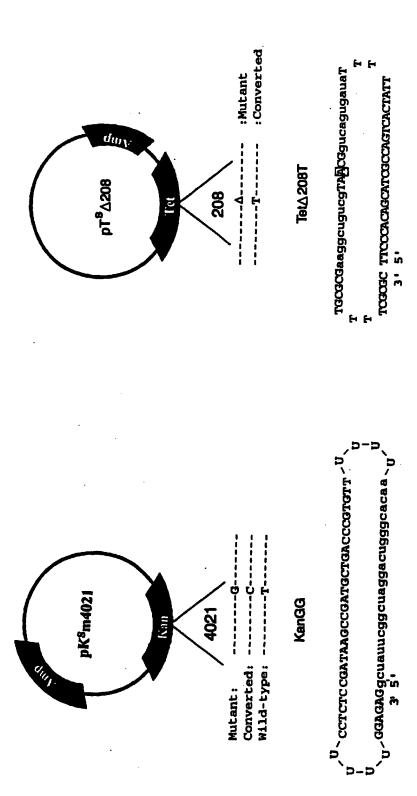
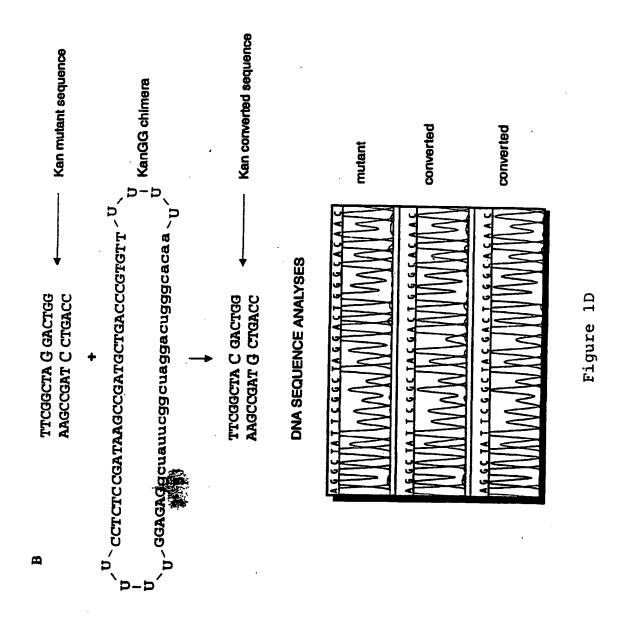


Figure 1C



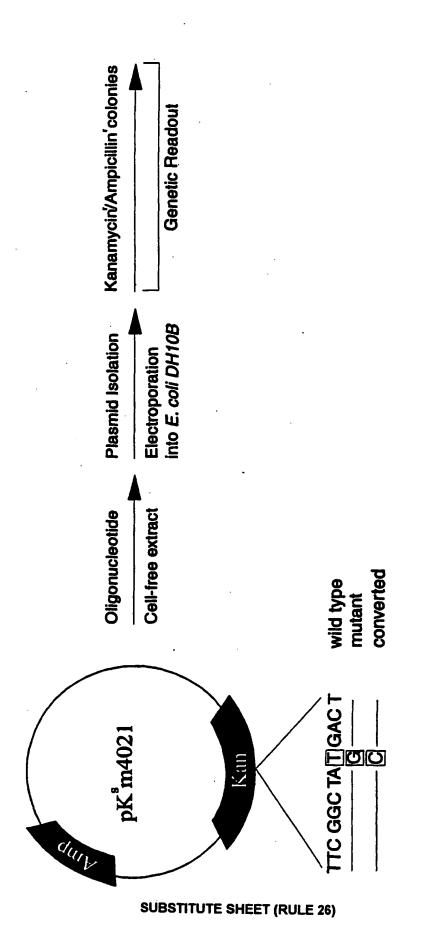
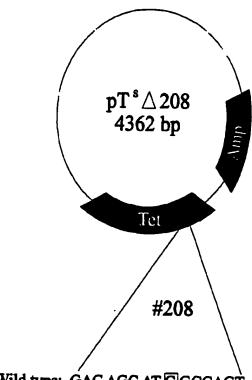


Figure 2



Wild type: GAC AGC AT CGCCAGT
Mutant: GAC AGC AT GCCAGT
Converted: GAC AGC AT TGCCAGT

## Sequence analysis of Tet<sup>r</sup> plasmid $\triangle 208$

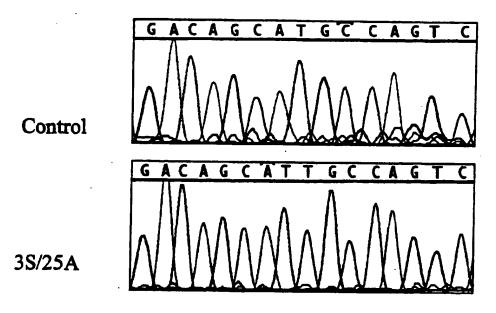


Figure 3

**SUBSTITUTE SHEET (RULE 26)** 

# DNA sequence analysis of Kan<sup>r</sup> plasmids

Target codon distr	ibution				
<u>oligomer</u>	TAG	TAC	TAC/TAG	TGG	TCG
1) 3S/25G (20)		+		100	100
2) 6S/25G (20)		+			
3) 8S/25G (20)		·	_	****	
4) 10S/25G (18)		·			_
5) 25S/25G (4)		т		+(2)	+(2)
3) 23G 23G (4)			+(2)	+(2)	

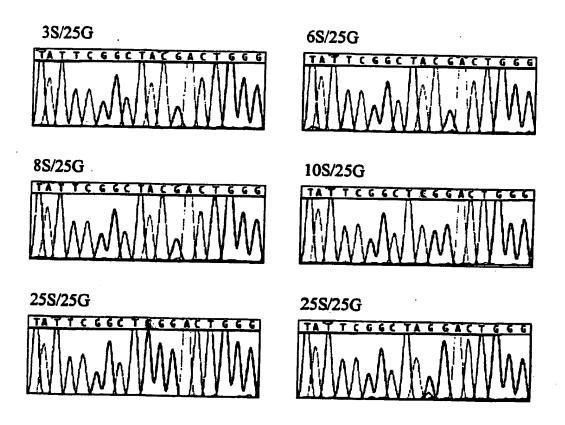


Figure 4

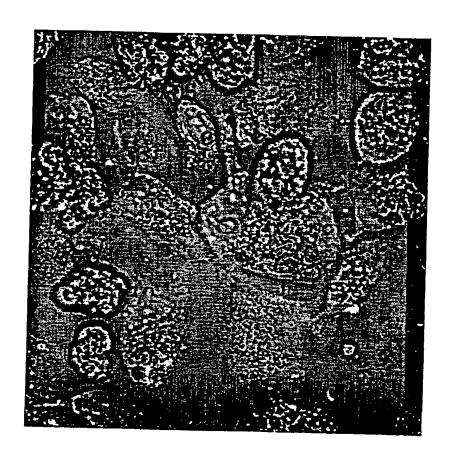


Figure 5

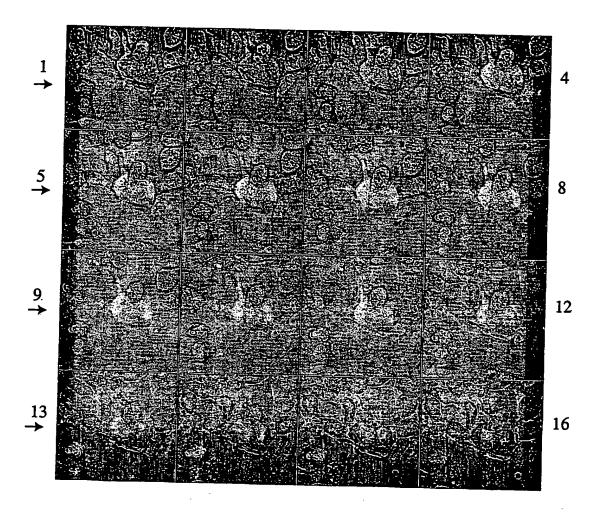


Figure 6

GTGGATATGTCCT Sequence of normal allele: Target/existing mutant: Desired alteration:

GTGGATAATGTCCT

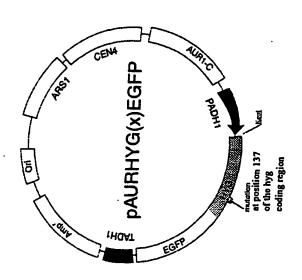
GTGGATACGTCCT

# Figure

Sequence of normal allele: GTGGATATGTCCT GTGGATAGGTCCT Target/existing mutant: Desired alteration:

GTGGATACGTCCT

Figure



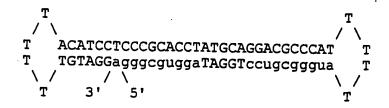
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HygE3T/25: 5'-AGG GCG TGG ATA CGT CCT GCG GGT A-3'

HVGE3T/74: 5'-CTC GTG CTT TCA GCT TCG ATG TAG GAG GGC GTG GAT ACG TCC TGC GGG TAA ATA GCT GCG CCG ATG GTT TCT AC-3'

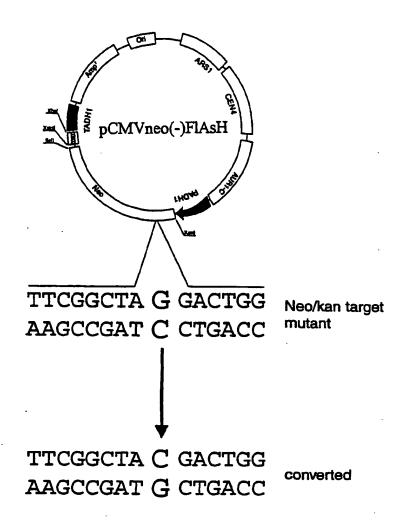
 $\frac{\text{HygE3T}/74\alpha:}{\text{CAG}}$  5'-GTA GAA ACC ATC GGC GCA GCT ATT TAC CCG CAG GAC GTA TCC ACG CCC TCC TAC ATC GAA GCT GAA AGC ACG  $\frac{\text{AG}}{\text{AG}}$ -3'

### HygGG/Rev:



Kan70T: 5'-CAT CAG AGC AGC CAA TTG TCT GTT GTG CCC AGT CGT AGC CGA ATA GCC TCT CCA CCC AAG CGG CCG GAG A-3'

### Figure 8



## FUSION GENE FOR LIGAND BINDING

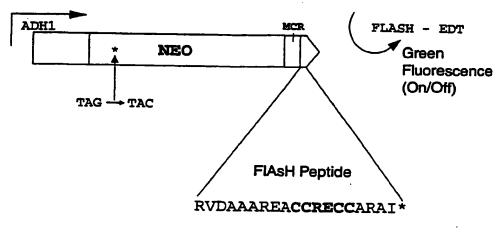


Figure 9
SUBSTITUTE SHEET (RULE 26)

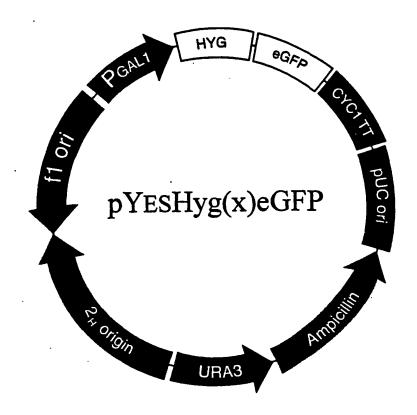


Figure 10

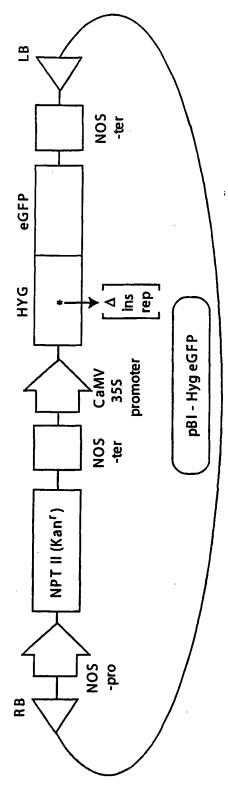


Figure 11

SUBSTITUTE SHEET (RULE 26)